CHEMICAL CHANGES DURING THE ENSILAGE OF GRASS WITH PARTICULAR REFERENCE TO CARBOHYDRATES

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ABSTRACT.

In the first section, the effect of delayed sealing on the biochemical changes occurring during ensilage was studied with particular reference to the fermentation of the carbohydrate fraction. The herbage was left uncovered for a period of 72 hours before it was covered and sealed off. The resulting 'silages' were analysed at this time and at the end of a normal ensiling period and they were compared with silages from silos which were sealed immediately. Losses of dry matter from the silos were measured and were compared for the two methods of ensilage. Delayed sealing had less effect on the course of fermentation in the first experiment in which the grass had a high water soluble carbohydrate content than in the second experiment in which the water soluble carbohydrate content of the grass was low. In this experiment, delayed sealing resulted in silages of higher pH values, butyrate and volatile-nitrogen contents and lower lactate levels. Dry matter losses were also significantly greater.

In the second section, the effect of formic acid on the fermentation pattern in grasses of different species and dry matters was investigated. Three experiments are described in which formic acid was added to low dry matter herbages. In the first and second experiments using timothy/meadow fescue, the levels of acid used were 0.22 per cent and 0.34 per cent respectively, whereas in the third experiment, using cocksfoot, the concentration of acid was 0.51 per cent. The immediate effect of the formic acid was to lower the pH values to 4.75, 3.81 and 4.11 in experiments F1, F2 and F3 respectively. Formic acid preserved water soluble carbohydrates in the early stages of harvesting and ensiling but did not prevent the formation of lactic acid in experiments F1 and F3. In all three experiments, the addition of formic acid did not reduce loss of dry matter and, in experiment F2 losses from the formic acid-treated herbage were higher than those from the control silages. This can be attributed mainly to the higher

effluent production when formic acid was used. The use of a high concentration of formic acid on the surface material in a silo is investigated in experiments F4 and F5. In experiment F6, formic acid was added to wilted perennial ryegrass (36 per cent dry matter) at the rate of 0.39 per cent. Changes during ensilage of this material were compared with changes occurring during ensilage of untreated wilted ryegrass and freshly harvested herbage. All silages were well preserved, of low volatile nitrogen content, and contained only traces of butyric acid. Formic acid restricted fermentation in the wilted grass, resulting in silage of high water soluble carbohydrate content (15.3 per cent) compared with untreated wilted (4.7 per cent) and fresh (1.2 per cent) silages. Results of microbial studies indicated that yeasts were more active in the formic acid-treated herbages. Surface waste production and fermentation plus oxidation losses were higher in the acid-treated wilted silages (21 per cent) than in the untreated wilted materials (14 per cent).

In the final section the day to day variation in water soluble carbohydrate content of ryegrasses was investigated. Random samples of grass were cut daily from replicated plots over 24-day periods in the early summer and in the autumn. The water soluble carbohydrate content of the grass showed a wide variation from day to day. The partial correlation coefficient of hours of sunshine and water soluble carbohydrate content, taking out a linear trend with time, was +0.744 in the early summer. In the autumn the relationship was weaker with the partial correlation coefficient = +0.343.

CONTENTS.

		Page
	Abstract.	i
I.	Introduction.	1
II.	Review of Literature.	2
	II. (1) Carbohydrate components of herbages and factors influencing them.	2
	II. (1) 1. Carbohydrate components.	2
	II. (1) 2. Factors influencing the carbohydrate content of herbages.	6
	II. (1) 2. 1. The growing plant.	6
	II. (1) 2. 1. (a) Species.	6
	(b) Variety.	8
	(c) Stage of growth.	10
	(d) Season.	11
	(e) Weather.	11
	(f) Fertiliser.	14
	(g) Time of day.	15
	(h) Management.	16
	II. (1) 2. 2. The harvested plant.	16
	II. (2) Changes in carbohydrates during ensilage.	18
	II. (3) The effect of oxygen on ensilage.	33
	II. (4) Formic acid as an additive for silage.	38
III.	Objects of study.	43
IV.	Techniques.	44
	IV. (1) Sampling.	44
	IV. (2) Silos.	44
	IV. (3) Losses.	46
	IV. (4) Methods of analysis.	47
	IV. (5) Bacteriological investigation.	50

		Page.
V.	The influence of delayed sealing on the chemical changes during ensilage.	52
	V. (1) Introduction.	52
	V. (2) Experimental.	53
	V. (3) Experiment 01 - Results.	54
	V. (3) 1. Temperature changes.	54
	V. (3) 2. Composition.	55
	V. (3) 3. Microbiological assay.	66
	V. (3) 4. Losses.	67
	V. (4) Experiment 01 - Discussion.	69
	V. (5) Experiment 02 - Results.	75
	V. (5) 1. Temperature changes.	75
	V. (5) 2. Composition.	75
	V. (5) 3. Microbiological assay.	87
	V. (5) 4. Losses.	89
	V. (6) Experiment 02 - Discussion.	91
VI.	Formic acid as a silage additive.	98
VI. a.	The effect of formic acid on the fermentation of grass of low dry matter and low water soluble carbohydrate content.	98
	VI.a.(1) Introduction.	98
	VI.a.(2) Experimental.	99
	VI.a.(3) Experiment F1 - Results.	101
	VI.a.(3) 1. Temperature changes.	101
	VI.a.(3) 2. Composition.	101
	VI.a.(3) 3. Microbiological assay.	105
	VI.a.(3) 4. Losses.	107
	VI.a.(3) 5. Laboratory silos.	109
	VI.a.(4) Experiment F1 - Discussion.	113

Page.

VI.a.(5) Experiment F2 - Results. 115

VI.a.(5) 1. Volume changes. 115

VI.a.(5) 2. Temperature changes. 115

VI.a.(5) 3. Composition. 116

VI.a.(5) 4. Losses. 120

VI.a.(5) 5. Laboratory silos. 123

VI.a.(6) Experiment F2 - Discussion. 127

VI.a.(7) Experiment F3 - Results. 128

VI.a.(7) 1. Volume changes. 129

VI.a.(7) 2. Temperature changes. 129

VI.a.(7) 3. Composition. 129

VI.a.(7) 4. Losses. 134

VI.a.(7) 5. Laboratory silos. 136

VI.a.(8) Experiment F3 - Discussion. 140

VI.a.(9) Experiments F4 and F5 - Results. 141

VI.a.(9) 1. Composition. 141

VI.a.(10) Experiments F4 and F5 - Discussion. 144

VI. b. The effect of formic acid on the fermentation of wilted grass. 146

VI.b.(1) Introduction. 146

VI.b.(2) Experimental. 147

VI.b.(3) Experiment F6 - Results. 148

VI.b.(3) 1. Volume changes. 148

VI.b.(3) 2. Temperature changes. 148

VI.b.(3) 3. Composition. 150

VI.b.(3) 4. Microbiological assay. 152

VI.b.(3) 5. Losses. 155

VI.b.(3) 6. Laboratory silos. 156

VI.b.(4) Experiment F6 - Discussion. 165

Page.

VII. The effect of sunlight on the water soluble carbohydrate content of ryegrasses. 170

VII. (1) Introduction. 170

VII. (2) Experimantal and Results. 170

VII. (3) Discussion. 176

VIII. Bibliography. 179

IX. Acknowledgements. 188

X. Appendix 1. - Methods of analysis. 189

XI. Appendix 2. - Results. 199

XII. Published Papers.

XII. (1) Effect of formic acid on the fermentation of grass of low dry matter content.

XII. (2) Chemical changes and losses during the ensilage of wilted grass treated with formic acid.

I. Introduction.

The importance of ensilage as a method of conserving grass for winter feed in countries with no winter growth and summer climatic conditions not always suited to successful hay making is now accepted. In the last 17 years in Scotland, the proportion of the total grass conserved which was made into silage rose from 10 per cent in 1965 to 34 per cent in 1972. With the recent steep rise in the cost of alternative sources of energy and protein, efficient silage making has an increasingly important rôle to play in the agricultural economy. In order to improve its quality, the processes involved in the production of silage must be fully understood. Although the importance of water soluble carbohydrates during ensilage has been realised for many years, it is only recently that modern analytical techniques have made it possible to identify the individual sugars present in grasses and silages and the products of their fermentation. The micro-organisms can be isolated and classified and the types of biochemical activity recognised. In the natural fermentation process sufficient water soluble carbohydrate must be present for the production of the lactic acid required to preserve the herbage. The amount of these carbohydrates available to the micro-organisms will be depleted to a greater or lesser extent by losses arising from oxidation by plant and microbial enzymes. The use of additives, such as formic acid, is currently an important economic factor in the conservation of herbage.

II. REVIEW OF LITERATURE.

II (I) CARBOHYDRATE COMPONENTS OF HERBAGES AND FACTORS INFLUENCING THEM.

II (I) I. Carbohydrate Components.

The carbohydrate components of grasses may be divided into two main groups (a) the non-structural carbohydrates and (b) the structural carbohydrates.

The former group, excluding starch which is insoluble in cold water, are commonly known as the water soluble carbohydrates (WSC) and are of great importance in the ensilage process because of their availability to the lactic acid bacteria. The WSC are further sub-divided into the monosaccharides, the oligosaccharides and the reserve polysaccharides.

The monosaccharides are colourless, crystalline solids, soluble in water and with a sweet taste. They are further classified according to the length of their carbon chain into trioses, tetroses, etc. In his review of carbohydrates, Percival (I952) claimed that the monosaccharides present in grass are glucose c. I per cent and fructose c. I-2 per cent. Xylose was tentatively identified in Trifolium pratense by Bailey (I958) and the sugar alcohol D - mannitol was reported in Lolium perenne by Harwood (I954). Beinhart (I964) found maltose in the stolons of Trifolium repens and Hansen etal (I958) suggested that small quantities of galactose may be present in some herbages.

The oligosaccharides are compound sugars containing between two and ten simple sugar residues. The most abundant of these in herbage is the disaccharide sucrose. Values of up to 7 per cent of the dry matter in Lolium perenne have been reported by Laidlaw and Reid (I952). Next to sucrose, raffinose is probably the most important oligosaccharide of the plant world. It exists in a free state in sugar beet from which it can be prepared on a commercial scale. Mild acid hydrolysis of raffinose yields D - fructose and melibiose, a disaccharide consisting of D - galactose and D - glucose. Enzymic hydrolysis yields sucrose

and galactose. Melibiose and the tetrasaccharide stachyose have also been found in grasses and legumes (Percival, 1952; Laidlaw and Reid, 1952; Hirst et al, 1959). Hunter et al (1970) were unable to identify raffinose and stachyose in the leaves and stems of tropical grasses (Cenchrus ciliaris, Chloris gayana, Cynodon dactylon, Digitaria decumbens and Sentaria sphacelata) but found them in legumes (Desmodium intortum, Desmodium uncinatum, Glycine javanica, Phaseolus atropurpurens and Stylosanthes humilis.

The third group of the non-structural carbohydrates contains the fructans, polysaccharides composed of fructose residues. In grasses these are levans, essentially linear molecules consisting of $2\rightarrow6^{1}$ linked β fructofuranose units terminating with sucrose residues (Hirst, 1959). They are amorphous or micro-crystalline, of varying solubility in water but insoluble in alcohol. The chain length is normally in the range 25-50 units but some short chain fructans (5-10 units) were identified by Harwood et al (1953). The fructans are temporary storage materials, by far the most easily hydrolysed of the polysaccharides, a hydrolytic breakdown occurring even in boiling water. In temperate grasses the fructans are the most abundant of the water soluble carbohydrates. Wylam (1953) showed that, after hydrolysis, the proportion of fructose: glucose: galactose in the stems of Lolium perenne was 100: 10: 1 due to the high proportion of short chain fructans. Because of their abundance and ease of hydrolysis by plant enzymes (Wylam, 1953), fructans are an important substrate for the lactic acid bacteria and normally disappear rapidly during the first few days of ensilage. They are also the WSC components most affected by environment, stage of growth and management (Mackenzie and Wylam, 1957; Jones et al, 1965, Nowakowski, 1962). The fructan content of grasses grown in warmer climates appears to be lower than that of grasses grown in cooler regions. Bathurst and Mitchell (1958) noted that the values they obtained for fructan content in New Zealand were

lower than those reported by Waite and Boyd (1953) for Scottish pasture grasses.

Fructans are not found in lucerne or clover where they are replaced by arabans and xylans with small amounts of polyglucans (Laidlaw and Reid, 1952). Hunter et al (1970) studying a range of tropical grasses and legumes, found that fructan was absent from all the species examined. The carbohydrate storage forms were mainly sucrose in the grasses and sucrose, starch and pectin in the legumes. Starch is not normally included under the term WSC because of its insolubility in cold water. It is not usually present in the stems or leaves of grasses grown in temperate regions although Bailey (1958) found 3 - 5.5 per cent starch in clover leaves compared with 0.7 per cent in ryegrass leaves. Other workers have reported starch in tropical grasses. Starch forms the main reserve polysaccharide in many other plants and is a mixture of two components, amylose and amylopectin. The proportions of the two components vary between plants but the majority of starches contain between 15 per cent and 30 per cent of amylose (Aspinall, 1970). Amylose contains linear chains of $1\rightarrow 4\alpha$ - D - glucopyranose residues, and amylopectin has a highly branched structure, the branch points involving $1\rightarrow 6^{1}$ linkages. A number of different α amylases have been characterised which convert amylose into maltose and glucose, attack being directed to non-terminal linkages. In contrast β amylases act on chains of $1\rightarrow 4^{1}\alpha$ - D - glucopyranose residues from the non-reducing end in a stepwise manner with the liberation of maltose only.

The second of the two main groups of carbohydrates contains the structural carbohydrates, cellulose, pectic substances and hemicelluloses.

Cellulose, the most abundant naturally-occurring organic substance is the principal cell-wall constituent of higher plants. The structural formulation of cellulose comprises linear chains of $1 \rightarrow 4^1$ linked β - D - glucopyranose residues containing 100 - 4,000 residues in a molecule and yielding only glucose on hydrolysis (Aspinall, 1970).

The term pectic substances refers to a group of complex plant polysaccharides in which D - galacturonic acid is the principal constituent and the term pectin is used in relation to gel-forming water soluble polysaccharides. Langston (1962) reported that glucuronic and galacturonic acids are readily utilised by silage micro-organisms. Although pectic substances are not an important constituent of grasses, Conrad et al (1958) found up to 8 per cent in Medicago sativa and Hirst et al (1958) reported values up to 10 per cent pectic substances in the leaves of the same species. Wright (1961) found 6 per cent in the leaves of Trifolium pratense.

The hemicelluloses in herbages have been reviewed by Aspinall and McGrath (1966) and Aspinall (1970). In general they are made up of $1 \rightarrow 4^1$ linked β - D - xylopyranose residues with single unit side chains. On hydrolysis they yield xylose, arabinose, and galactose. Arabans are found associated with pectic substances from a number of sources. Hydrolysis of hemicelluloses in the ensilage process is known to occur and Dewar (1963) showed that lactic acid bacteria can utilise pentoses. Harwood (1954b) reported the loss of pentosans during the ensilage of Lolium perenne when the pH value was near the neutral point. The major constituents of the WSC content of silages can be the breakdown products of hemicelluloses (Bousset, 1967).

II (1) 2. Factors influencing the carbohydrate content of herbages.

II (1) 2. 1. The growing plant.

II (1) 2. 1. (a) Species.

Fructan is the main component of the WSC in temperate grasses and is the most variable constituent, responsible to a great extent for the differences among the species. Lolium multiflorum has the highest WSC content among the grasses, values up to 38 per cent of the dry matter having been reported (ap Griffith, 1962). At the other end of the scale the legumes contain little or no fructan and are lower in WSC content than the grasses. The published results of the analysis of a large number of samples of grasses grown in Great Britain are shown in Table I.

TABLE I

WSC contents of grasses (per cent in DM)

Species	Samples	Range	Mean	SE
Lolium multiflorum	38	7.4 - 31.4	18.1	± 0.96
Lolium perenne	191	4.6 - 31.5	17.0	± 0.38
Phleum pratense	20	5.3 - 19.9	11.0	± 1.07
Festuca pratensis	17	3.5 - 26.3	9.6	± 1.44
Dactylis glomerata	107	0.5 - 19.1	7.9	± 0.41

Waite and Boyd (1953b) studied Lolium perenne (S23), Dactylis glomerata (S143), Festuca pratensis (S53) and Phleum pratense (S48) and found Lolium perenne to have the highest WSC content, followed by Phleum pratense, Festuca pratensis and Dactylis glomerata. Cooper (1961) reported that, although some overlapping did occur, the W.S.C. content of seven varieties of Dactylis glomerata were lower than those of eight varieties of Lolium perenne.

McDonald and Henderson (1965) analysed five species sown in randomised blocks and cut at two stages of growth. At each cut Lolium multiflorum had the highest WSC content. At the first cut Phleum pratense had the lowest WSC content and at the second cut Dactylis glomerata had the lowest (Table II).

TABLE II

DM and WSC contents of five grasses cut on 5 Aug. and 16 Sept.

Species and variety	Cut 1		Cut 2	
	% DM	% WSC in DM	% DM	% WSC in DM
Lolium multiflorum (Hinderupgaard)	15.2	18.6	14.0	11.0
Lolium perenne (New Zealand)	14.8	12.0	13.2	6.3
Phleum pratense (Scots)	18.5	8.7	13.4	4.1
Festuca pratensis (S215)	16.7	8.9	14.9	4.5
Dactylis glomerata (Scotia)	15.2	9.3	12.9	3.9
LSD (P = 0.05)	0.98	1.04	1.09	1.38

Oehrung (1963) concluded from experiments carried out over three years that the suitability of the most important species of grasses for silage making increased from Dactylis glomerata and Phleum pratense, through Festuca pratensis and Lolium perenne to Lolium multiflorum. He ascribed the differences in suitability of the grasses to their WSC contents. Witt (1967, 1970) and Sevenster (1970) published similar results.

Witt (1970) and McDonald and Henderson (1965) noted that the WSC content of legumes was low relative to ryegrasses. The results of the analysis of six varieties of clover harvested in June and August are given in Table III.

TABLE III

DM and WSC contents of six clovers cut on 10 June and 27 Aug.

Species and variety	Cut 1		Cut 2	
	% DM	% WSC in DM	% DM	% WSC in DM
Late flowering red, Altaswede	9.9	10.8	16.6	5.3
Late flowering red, Montgomery	10.9	9.6	16.6	5.6
Broad red, English	11.6	8.7	18.4	6.5
Broad red, Essex	10.5	10.4	17.4	5.3
White, S100	12.7	7.1	17.6	5.1
White, New Zealand	10.8	9.1	15.6	5.6
LSD (P = 0.05)	0.58	1.52	0.58	0.81

Davies et al (1966) found that the WSC content of Trifolium repens was higher than that of Trifolium pratense, which in turn was higher than that of Medicago sativa.

II (1) 2. 1 (b) Variety.

Within any species varietal differences in WSC are known to occur. Cooper (1961) found that the continental pasture varieties of Lolium perenne, Melle and Pajbjerg were consistently high in WSC content, while Algiers was low. The range was greater in Dactylis glomerata, with the continental varieties Russia and Danish highest and S143 and Portuguese diploid much lower. Reveille and Petra, tetraploid varieties of Lolium perenne were shown by Aldrich and Dent (1963) to have consistently higher WSC contents than the diploid varieties.

The superiority of Reveille as a source of WSC has been confirmed by several workers. McDonald and Henderson (1963) analysed 15 diploid varieties

and one tetraploid (Reveille) of Lolium perenne at monthly intervals over a season and found the tetraploid to have the highest WSC (Table IV). The mean value for the season was significantly higher (P = 0.05) than the mean of the other varieties and significantly higher than the value for any other variety.

TABLE IV

WSC content of 16 varieties of Lolium perenne (% DM)

Variety	Cut 1 1 May	Cut 2 31 May	Cut 3 1 July	Cut 4 31 July	Cut 5 29 Aug	Cut 6 2 Oct
N.Z. Mother seed	14.5	19.5	14.3	18.2	20.8	13.6
Hooiberg	15.7	22.1	13.8	18.0	20.4	13.6
Steinacher	13.7	21.6	14.5	20.5	21.5	14.1
Early Trifolium Elite	14.1	23.0	14.7	18.6	21.7	14.1
Primavere	14.5	21.6	16.3	19.8	22.1	15.6
Stormont L	15.4	20.9	16.8	20.6	21.5	16.7
Sceempter Hay Type	11.6	19.9	15.4	20.7	18.5	16.4
Pajbjerg Verna III	13.0	22.5	15.4	19.6	20.0	15.1
Oregon Blue Tag	14.9	21.6	15.6	19.1	21.1	15.1
Early Otofta III	14.4	20.1	16.0	19.7	17.9	15.2
Delta	14.4	24.1	15.6	18.4	18.0	14.5
Reveille	17.7	23.3	19.8	23.0	25.5	17.2
Linn	13.2	21.1	15.1	20.4	20.1	14.6
Stormont E	15.7	17.9	15.4	19.0	21.2	15.4
Combi	13.7	21.7	16.7	21.2	21.3	15.0
Mommersteegs Early Hay	14.8	23.3	16.2	20.9	20.6	14.3

Castle and Watson (1971) compared diploid and tetraploid ryegrasses as herbages for silage production and concluded that silages made from Reveille were superior due to the higher WSC content of this variety.

Dent and Adrich (1963) found that early varieties of Dactylis glomerata had consistently higher WSC contents than the late varieties. Varietal differences were also found in Festuca pratensis and Phleum pratense by these workers. Davies et al (1966) found little evidence that the polyploid clovers were higher in WSC content than the diploid forms.

II (1) 2. 1 (c) Stage of growth.

Waite and Boyd (1953a)cut Lolium perenne at weekly intervals during the growth cycle from April to October on two successive years. Mackenzie and Wylam (1957) cut grass at two-weekly intervals from May until early November. The results of these experiments confirmed that there were no seasonal trends in the monosaccharides or sucrose content. Mackenzie and Wylam found that the fructan showed an initial increase which was followed by a sudden decrease in early June, a subsequent increase reaching a maximum value in September preceding a further fall. Waite and Boyd found only one peak for the fructan content of Lolium perenne and two for Festuca pratensis, Dactylis glomerata and Phleum pratense. ap Griffith (1962) studied the chemical composition of eight grasses at intervals during primary growth (March to June) and noted that, in general, the eight grasses were at their highest level of WSC content during May and early June, but the actual peak was not reached at the same date in every case.

Hirst et al (1959) studied the WSC content of Medicago sativa (du Puits) over two seasons. During the first year of growth there was an accumulation of sucrose. During the second year the WSC content was highest at the flowering stage. The work of Melvin (1965), Bailey (1964) and Davies et al (1966) confirmed this finding.

Jones (1970) studied the cell wall constituents of some grass species and found that the structural carbohydrate content increased with maturity. At the same stage of digestibility Dactylis glomerata had a lower structural carbohydrate content and higher lignin content than other species. Waite and Gorrod (1959) investigated five grasses at four stages of growth and observed that xylan, cellulose and uronic acid contents increased appreciably with the age of the plant. Pressey et al (1963) found that total pectic substances increased gradually throughout the growth cycle.

II (1) 2. 1 (d) Season.

To simulate intensive grazing, Waite and Boyd (1953b) cut four grasses each time they reached 8 - 10" during the growing seasons of 1951 and 1952. They found that grass in which the growing point had recently changed to floral development (May - June) contained more WSC than grass grown and cut later in the season. In the latter part of the growing season WSC contents increased again but the grasses were more leafy and contained much less fructan in the stems than at earlier growth stages. McDonald and Henderson (1963) cut 16 varieties of Lolium perenne at monthly intervals from May to October (Table IV). The values for all the varieties followed the same seasonal trend and maximum WSC contents were recorded in late May and late August. Differences in fertiliser treatment and/or weather may have accounted for the high WSC values obtained later in the season by McDonald and Henderson but not by Waite and Boyd.

II (1) 2. 1 (e) Weather.

Many variable factors come under this heading and it is difficult to study any one in isolation in the field. Most of the work reported in the literature has been confined to the study of the effects of temperature and light intensity on WSC under controlled environmental conditions in greenhouses. The problems of relating the results of such studies to field work were discussed by

Alberda (1965a) who claimed that climatic control gives valuable information concerning the influence of separate factors on biochemical processes such as development, DM production, and chemical composition that would not be possible otherwise.

Alberda (1957) reported on an experiment in which Lolium perenne was grown in greenhouses where temperature and light intensity were controlled. At the medium light intensity (22,000 ergs/cm^2/sec) the rate of soluble carbohydrate production was less than half of that at the high light intensity (41,000 ergs/cm^2/sec), while at the low light intensity (12,000 ergs/cm^2/sec) there was almost no carbohydrate formation. Low night temperatures (3°C) led to higher WSC contents than did high temperatures (28°C) at the same light intensity. In a later paper, Alberda (1965b) stated that WSC content was always higher at the higher light intensity, independent of temperature but, as respiration increases considerably with temperature, WSC content was higher at the lower temperatures at any one light intensity. Deinum (1966) cut Lolium perenne after a growth period of four weeks at three light intensities and three temperature levels. DM production, DM percentage and WSC content increased at the higher light intensities. WSC content was always highest at the lowest temperature. The results of this experiment were considered to agree well with the composition of grasses grown in the field from spring to midsummer, and to show that the chemical composition of grass in the field is considerably influenced by light intensity and temperature in temperate regions.

Bathurst and Mitchell (1958) showed that different levels of light and temperature were accompanied by very great differences in some of the constituents of grasses and clovers when they were grown in greenhouses and out of doors. WSC contents were highest with full light and low temperature and lowest with shade and high temperature with the exception of Trifolium repens

grown outside. Bowden et al (1968) reported no relationship between temperature at time of cutting and the WSC content of Dactylis glomerata. Waite and Boyd (1953a) noted that relatively small climatic differences caused considerable change in the rate and type of growth (vegetative or floral) with a consequent two-fold or three-fold variation in the WSC values. Differing patterns in the WSC contents for two consecutive years were explained by differences in weather conditions. Mackenzie and Wylam (1957) recorded a marked decrease in the total soluble sugar content of Lolium perenne grown in the field during the period of initial rapid growth from 1 to 17 June. This coincided with a period of little sunshine and they considered that the low WSC content may have resulted, in part at least, from this. When the same workers covered half a plot with hessian to simulate conditions on a dull day, the sucrose content fell off rapidly during the first day. The fructan content remained the same in both plots after one day, but fell off slightly after two days. There was no further change in the covered plots after four days. When part of the covered plot was uncovered on the second day the sucrose content rose sharply. When the sucrose content of the whole plant was correlated with sunshine the sucrose peaks coincided with maximum amounts of sunshine on the day before cutting. Myhr and Soebø(1969) reported that the WSC content of some grass species was almost halved by shading. Witt (1967) stated that the weather a few hours before harvest had a great influence on the WSC content. The same worker (1970) claimed that, apart from the first week after cutting, the climatic conditions may have a more important influence on the WSC content of grasses than the stage of growth. Under bad weather conditions he found the WSC contents of ryegrasses to be almost as low as those of legumes.

II (1) 2. 1 (f) Fertiliser.

The very marked effect of nitrogenous fertilisers on the WSC content of grasses is associated with the change in fructan content. The application of these fertilisers encourages a quick, leafy growth and produces a grass with a much higher leaf: stem ratio than the untreated grass. This stimulation of leaf growth is at the expense of the temporary storage polysaccharide, fructan (Nowakowski, 1969).

Jones et al (1965)studied the effect of nitrogenous fertilisers on the WSC contents of pure grass swards. Sodium nitrate markedly depressed the WSC content of Lolium perenne and Dactylis glomerata during primary growth. In a second experiment the decrease in WSC content was found to be less with urea as fertiliser than with ammonium sulphate or sodium nitrate. Sprague and Sullivan (1950) demonstrated that a high application rate of nitrogenous fertiliser increased the utilisation of fructan in the stubble of Dactylis glomerata.

Nowakowski (1969) noted that, with increasing applications of potassium fertiliser, there is an increase in fructan content. Cooil and Slattery (1948) claimed that adequate potassium was required to polymerise fructose to fructan.

Russel (1938) observed that phosphorus deficiency increased fructan levels through its effect in restricting vegetative growth.

Waite (1957) studied the effect of a compound fertiliser (N - 12 per cent; P - 4 per cent; K - 12 per cent) on the WSC content of five grasses. Increase in fertiliser application led to decrease in WSC content in all species. As the efficiency of the fertiliser in raising the nitrogen content of the grass increased, sucrose replaced fructan as the main component of the WSC fraction except in the slow growing Lolium perenne (S23).

II (1) 2.1.(g) Time of day.

Waite and Boyd (1953a) studied the diurnal variation of the WSC fraction of Lolium perenne and found that the sucrose content rose to a maximum in late afternoon (15.00 h - 18.00 h) while the hexoses fell to their lowest at that time and the fructan content varied irregularly. De Man and de Heus (1949) found sucrose to be the only sugar to show a distinct diurnal variation. Mackenzie and Wylam (1957), working with Lolium perenne grown under sunny conditions, found that the sucrose content rose to a maximum during the early afternoon and fell off sharply at sunset.

McDonald and Henderson (unpublished data) cut Dactylis glomerata at two-hourly intervals from 08.00 h to 16.00 h in sunny and in overcast conditions. On each occasion the mean value of the WSC content of four plots cut was highest at 12 noon but the variation among the plots at any one cut was too great to demonstrate any statistically significant variation (Table V). Similar results were obtained with Lolium perenne, with the maximum occurring at either 12.00 h or 14.00 h (McDonald and Henderson, 1966).

TABLE V.

Diurnal variation of WSC content in Dactylis glomerata.

Time of Day	Sunny		Overcast	
	% WSC in DM	Range	% WSC in DM	Range
08.00 h	7.5	7.1 - 8.3	6.7	5.8 - 7.6
10.00 h	7.2	5.4 - 10.6	6.8	6.5 - 7.1
12.00 h	9.0	7.4 - 9.9	7.5	6.6 - 8.1
14.00 h	8.2	7.9 - 8.7	6.5	5.9 - 7.1
16.00 h	8.8	7.8 - 10.2	6.5	5.1 - 7.1

Holt and Hilst (1969) found that the WSC content of Medicago sativa followed a curvilinear diurnal trend from low at 06.00 h to a maximum at noon, decreasing to 18.00 h. Non-structural polysaccharides followed a non-linear daily trend with the most rapid increase in the afternoon. The grasses examined underwent linear increases in WSC and non-structural carbohydrate contents. Melvin (1966) found that the soluble sugar and starch contents of Medicago sativa were higher in the afternoon than in the morning. Bowden et al (1968) noted that Dactylis glomerata cut at 16.00 h contained 3 per cent units more WSC than that cut at 09.00 h. WSC contents decreased overnight in most plants. Similar results were published by ap Griffith (1965), Davidson and Milthorpe (1965) and Eagles (1967).

Pressey et al (1963) found that pectins increased during the day and decreased during the night, but changes were small.

II (1) 2. 1 (h) Management.

Frequency of cutting and extent of defoliation are known to influence the carbohydrate content of plants. With grasses clipped every two to three weeks, Waite and Boyd (1953b) noted a marked decrease in fructan content.

Haydon (1970) observed that plants established a reserve of sugar and starch between the 50th and 80th days. Regrowth was poorer when plants were defoliated before this.

II (1) 2. 2. The harvested plant.

Wylam (1953) working in the laboratory found that 2 h after harvesting the WSC content had fallen from 18.8 per cent to 17.3 per cent and the fructan content from 9.6 per cent to 9.2 per cent. After 24 h there was a further fall to 15.4 per cent and 5.2 per cent respectively.

In two experiments carried out in the field using ryegrass/clover mixtures wilted under poor weather conditions, McDonald and Henderson (unpublished data) found that the WSC content fell from 7.0 per cent to 5.5 per

cent after 9 h in the first experiment and from 16.7 per cent to 13.4 per cent after 5 h in the second experiment.

In the first of two other experiments using Lolium perenne, open to the light and completely shaded, McDonald and Henderson (unpublished data) found that the WSC content rose from 10.1 per cent to 10.2 per cent after 24 h in the open but fell to 9.4 per cent after 48 h. In the second experiment, weather conditions were better and the WSC of the grass open to the light rose 3 per cent units after 72 h and remained constant in the shade. Stone et al (1943) and Dexter (1945) quoted figures showing increases in WSC content on wilting.

Pizarro and James (1972) observed that respiration declined with decreasing moisture content. The younger the plant material, the greater was its rate of respiration and therefore respiration losses were relatively more important as a factor depleting WSC in young herbage. Weight losses were small and amounted to only 1 per cent of the dry matter in their experiments. In terms of WSC this loss was quite high.

Some sugar loss due to respiration will be made up by photosynthesis. MacGregor (1966) noted that photosynthetic activity during a 24 h wilting period in dull weather was 98 per cent of that of the growing plant. During the ensuing 72 h, long periods of sunshine increased the grass dry matter to 68 per cent and photosynthesis rapidly decreased. Even after 48 h of wilting, photosynthetic activity can be quite extensive.

Mechanical treatment of the herbage after harvesting will affect respiration and photosynthesis. Frequent turning of the swath and rapid drying will minimise respiration. Pizarro and James (1972) found that density of the swath affected photosynthesis as self-shading did occur. Delay between lifting of the grass in the field and consolidation of the grass in the silo may also lead to a considerable decrease in WSC content.

II. (2) CHANGES IN CARBOHYDRATES DURING ENSILAGE.

It has been accepted since the end of the last century that production of silage depends upon chemical changes involving the sugars of the ensiled material. Russell (1908) listed the changes taking place during the ensilage of maize as the disappearance of sugar, the formation of volatile fatty acids and the hydrolysis of protein. Lamb (1917) recognised the relative influence of micro-organisms and plant enzymes on fermentation during the ensilage of corn and stated that the disappearance of simple sugars with the production of carbon dioxide, alcohols and acids and the degradation of protein are the chemical phenomena of the fermentation. He noted that carbon dioxide production was greatest during the first day of ensilage, decreasing thereafter. In laboratory experiments, where there was little chance of inoculation of the herbage, acid production was slow and sugars increased during the first day of ensilage.

From this early work until the present day research has continued into the microbiology and biochemistry of ensilage and now the strains of bacteria present can be identified and many of the pathways by which carbohydrates are converted into acids and other byproducts can be followed in detail (Langston et al, 1958; Beck, 1966; 1969; Whittenbury et al, 1967; Honig, 1968).

The changes occurring during the ensilage process are dependent upon the conditions prevailing during ensilage, the plant enzymes, bacteria, yeasts, etc. present, and upon the nature of the substrate available to them. The carbohydrates which are present in the plant have been discussed in the previous section. Salisbury et al, (1949) determined the relative amounts of acid produced by mixed cultures of silage organisms when offered various carbohydrates as sources of energy and found that arabinose, fructose, glucose, sucrose and xylose produced more acid than galactose, rhamnose, lactose, inulin,

dextrin, soluble starch and several sugar alcohols. Lanigan (1966) stated that none of the silage strains of Lactobacillus brevis, Lactobacillus plantarum and Pediococcus sp. isolated, utilised fructans as an energy source but it must be assumed that as these usually disappear during ensilage fructans must be readily hydrolised and thus made available to the silage organisms.

Archibald (1953) noted that in most cases sugar equivalent in the silage in the form of aliphatic acids (acetic, butyric and lactic) was greater than could be accounted for by the sugar content of the freshly ensiled crop. The work of Langston et al, (1962), showed that when sugar levels were low, other substrates were available for bacterial metabolism. Various workers have produced evidence that pentosans and galactan are fermented by lactic acid bacteria (Harwood, 1954; Macpherson et al, 1957; and de Man, 1957). Dewar et al, (1963) were unsuccessful when they attempted to grow a number of strains of lactic acid bacteria using hemicellulose as an energy source, but they did produce appreciable amounts of reducing sugars both by enzymic breakdown of hemicellulose and by the acid hydrolysis of hemicellulose at pH. 4.

The results of three experiments reported by McDonald et al, 1960, indicated that considerable breakdown of hemicellulose occurs even in well preserved silage where dry matter losses are low. Gouet and Fatianoff, (1964) attributed the increase in concentration of reducing sugars with certain treatments to the plant enzyme hydrolysis of hemicellulose. Bousset, (1967) compared the compositions of hemicellulose before and after ensiling and found them to be very different due to the release of side-chains of the hemicellulose by plant and/or bacterial enzymes.

Attempts have been made by several workers to increase the soluble sugar content of crops by the addition of enzymes. Lamb (1917) suggested that the increase in sugar content during the first day of ensilage of corn was due to the presence in the corn grain of amylases which produced sugar from the higher

carbohydrates faster than the sugar was used up by bacteria. Lyford (1969) noted a significant increase in cellulase activity at the onset of silage fermentation, reaching a peak on the second day and continuing high until the 14th day. Rydin (1961) stated that the beneficial effect which a mixture of malt meal and wheat meal had on the ensilage processs was a result of the enzymes present in the malt meal. These catalysed the hydrolysis of raw starch in the meal mixture and of certain polysaccharides in the green forage into fermentable dextrins and sugars for lactic acid bacteria. McCullough (1970) reported on the addition of amylase in the form of malted barley or as a crude preparation of the enzyme. Both acetic and lactic acids were increased in the silages by the addition of one per cent calcium carbonate with the enzyme, but the silages were consumed in smaller quantities than the control silages with a loss in milk production. Research is continuing into the use of amylase enzymes. Svensson and Tveit (1964) added an amylase preparation to hydrolyse the polysaccharides of plant material of low sugar content but this was unsuccessful.

The initial breakdown of carbohydrates during the ensilage process is due to respiration. The soluble carbohydrates with the residual oxygen in the silo are converted to carbon dioxide and water with the production of heat. This will be discussed more fully in the next section.

According to Barnett (1954) respiration can proceed under anaerobic conditions in the presence of phosphate with the production of pyruvic acid, lactic acid, acetaldehyde, ethanol and carbon dioxide. The destruction of carbohydrate by plant enzymes will cease when acid conditions prevail in the silo.

Hunter (1921) showed that, in the main, micro-organisms are responsible for the production of acids in silage and Heineman and Hixson (1921) stated that

there are three stages in the bacterial fermentation in silage:-

(1) Fermentation by the coliform group whose chief product is acetic acid.

(2) Fermentation by streptococci, producing small quantities of acetic and lactic acids.

(3)Fermentation by lactobacilli, producing lactic acid.

Knowledge of the microflora on the plant material to be ensiled gives little indication of the changes which will take place in the silo. Most of the investigators studying fermentation of grass have shown that coliform and pigmented organisms are present in relatively large numbers on fresh forage. Allen et al (1937) observed a marked change in the microflora after a very short period in the silo, the lactobacilli having multiplied. Cunningham and Smith (1939) also observed that the microflora of the plant material shortly after collection into the silo was entirely different from the microflora of the corresponding fresh plant material. Stirling (1953) stated that the counts of lactobacilli on the fresh material may not always be sufficiently high to ensure preservation when the fodder is made into silage. Weise (1969) found that the lower the number of lactic acid bacteria on the herbage, the higher was their rate of growth in the silo.

Hunter (1921) studied the microbial activity in lucerne silage. The unfavourable fermentation in the control silage was changed to a favourable one by the addition of fermentable carbohydrate to the lucerne but he found no difference in the microflora of the two types of silage. Kempton and San Clemente (1959) observed that spoiled silages contained the same number of lactic acid bacteria as the equivalent well-preserved silages at every stage of fermentation. Lanigan (1966) found that similar counts of lactic acid bacteria are usually obtained in materials that ultimately produce both good and bad silage and stated that the nature of the substrate remaining for the stationary-phase lactic acid bacteria and other species present is of greater importance

to the outcome of ensilage.

Detailed characterisation of silage microflora has been carried out by many workers. Cunningham and Smith (1939) investigated the organisms which survived on silage made with A.I.V. acid and found them to be mainly lactobacilli, streptococci, micrococci and sarcinae. Some samples also contained yeasts and yeast-like organisms. Both homo- and heterofermentative bacteria were found among the lactobacilli. Streptococci and micrococci were most numerous in fodder recently ensiled. Lactobacilli and sarcinae were associated with older samples. Virtanen (1937) indentified a long rod (Lactobacillus) in fodder crops treated with mineral acids. This produced lactic acid from both hexoses and pentoses. The presence of acetic acid in the early stages of ensilage was attributed to the activity of Lactobacillus pentoaceticus, known now as Lactobacillus brevis. Virtanen also noted the presence of yeasts. Counts of clostridia, almost the only micro-organism responsible for destroying anaerobic silage, have generally been found to be quite small (Allen and Harrison, 1937; Gibson et al, 1958), Bhat and Barker, (1947) discussed the rôle of acetic acid in the butyric acid fermentation of lactate. This fermentation normally occurs at a later stage in silages made from herbage with an inadequate carbohydrate content. Certain clostridia have been shown to multiply at an early stage, almost as early as do other silage organisms (Gibson et al, 1958). McDonald, et al, (1966) detected them in ~~in~~ concentrations of up to 100,000/g during the first few days of ensilage of a high moisture crop which reached a mean temperature of 42°C on the third day. Clostridium tyrobutyricum ferments lactate to butyric acid but Clostridium butyricum is known to ferment sugars to butyric acid and carbon dioxide, accompanied by a high loss in dry matter (Allen and Harrison, 1937).

Nilsson (1956) stated that it is not sufficient to know the micro-organisms present on a herbage but the environment in the silo must be known also if the

course of fermentation is to be predicted.

Stirling (1951) studied the effect of temperature and wilting and chopping of herbage on bacterial numbers and acid production. The bacterial population reached a peak earlier in material held at 30°C but counts tended to be higher at 22°C. Wilting delayed bacterial action while chopping hastened the action and helped to ensure a low pH. Gibson et al, (1961) found that the growth of gram negative organisms and the main groups of lactic acid bacteria was fastest on minced material held at 30°C in laboratory test-tube silos. Large scale experiments proved that laceration of the herbage had a beneficial effect on the bacterial population.

Nilsson et al, (1956) studied the rôle of temperature on the ensilage process and found that the production of lactic acid took place more rapidly at higher temperatures. Most of the acid was formed in the first week of ensilage. When high protein, low sugar crops are ensiled, the temperature is an important factor, greatly influencing the quality of the silage. When an 85 per cent clover, 15 per cent grass mixture was held at 5°C the pH value was 4.9 after one week and 4.7 after four weeks. When the same mixture was held at 37°C the pH value of the silage was 4.2 after one week but had risen to 5.4 after four weeks. The higher temperature led to stronger bacterial activity and protein breakdown accompanied by a pH rise. The low sugar content was insufficient to produce adequate amounts of lactic acid. Lanigan (1965) stated that Lactobacillus brevis, Lactobacillus plantarum and pediococci emerge as the dominant population of silage. At temperatures above 36°C pediococci become increasingly predominant and above 44°C it is rare to find lactobacilli. The growth and acid production of Lactobacillus plantarum are greatest within the range 30° - 35°C and all strains of pediococci grow well at 44° - 45°C. Wieringa (1959) concluded that the laceration of grass had the same effect on silage fermentation as the addition of sugars but if the crushed grass is held at 30°C, butyric acid

bacteria may benefit from the better availability of the sugars. A low temperature is just as important for the preservation of lacerated grass as it is for the preservation of untreated grass.

Wieringa (1958) showed that the great value in wilting is in the rise in osmotic pressure which accompanies it and which inhibits the development of butyric acid bacteria. The resistance of clostridia to osmotic pressure depends on the pH value of the medium. With an increase in osmotic pressure butyric acid production ceases, even at a high pH value. Wieringa (1960) stated that the bacterial flora exercise a much greater influence on the process of fermentation in wet grass than does its chemical composition. Their importance is less in the case of wilted grass in which undesirable types of bacteria are inhibited.

Little emphasis has been laid on the changes in carbohydrates due to yeast fermentation with the production of ethanol and carbon dioxide. Pedersen (1967) analysed 129 samples of grass silage and 23 samples of beet-top silage and found an average of 1.18 per cent ethanol in the dry matter of grass silage and 0.42 per cent in the dry matter of beet-top silage. Under certain conditions the ethanol content of silage may be well above the average. When bacterial activity was almost completely inhibited by the addition of 2 per cent tartaric acid (Lamb 1917) the ethanol content was about six times higher than that of the control silage. This high value was attributed to yeasts being favoured under these conditions.

In conclusion, the principal changes in carbohydrates during the ensilage process are summarised below -

(1) Aerobic respiration to carbon dioxide and water.

(2) Anaerobic respiration to pyruvic acid, lactic acid, acetaldelayde, ethanol and carbon dioxide. This is described by Stanier et al, (1963) as an energy-yielding biological oxidation in which an inorganic substance other than oxygen, namely nitrate, sulphate or carbonate is used as the external electron acceptor.

(3) Fermentation by the coliform bacteria (e.g. Escherichia coli)to formic acid, acetic acid, lactic acid, succinic acid, ethanol, carbon dioxide and hydrogen (Stanier et al, 1963). A typical fermentation of glucose by Aerobacter aerogenes produces 2, 3 butane-diol in addition to the above products. The fermentation of glucose by Escherichia coli is shown in Figure I (Wilkinson and Rose, 1963).

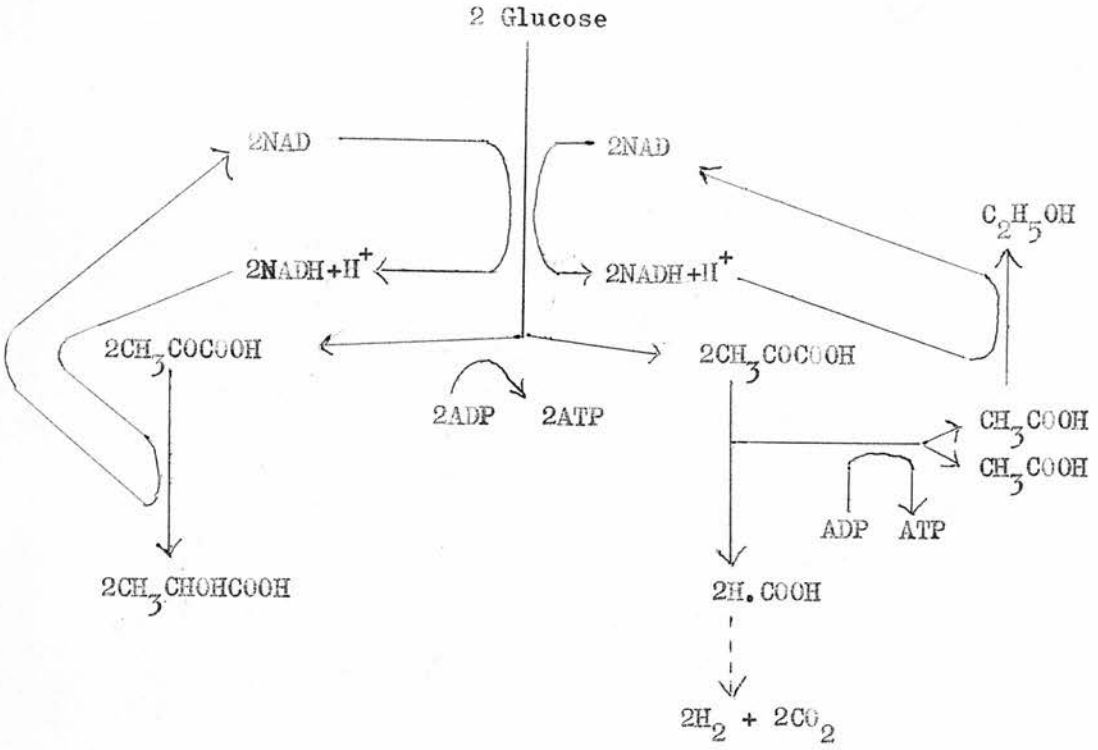
(4) Fermentation by lactic acid bacteria of the homofermentative and heterofermentative forms. The homolactic fermentation of glucose and fructose is shown in Figure II. Lactic acid is virtually the sole end product of glucose and fructose breakdown. The heterofermentative lactic acid bacteria produce additional end products from glucose (carbon dioxide, ethanol and sometimes acetic acid) and from fructose (carbon dioxide, mannitol and acetic acid). The heterolactic fermentation of glucose, fructose, arabinose and xylose are given in Figures III, IV and V respectively.

(5) Fermentation by clostridia e.g. Clostridia butyricum resulting in the formation of butyric acid, acetic acid, carbon dioxide and hydrogen. The clostridial fermentation of glucose is shown in Figure VI.

(6) Fermentation by yeasts to ethanol and carbon dioxide.

In a sealed system aerobic respiration will be over in two hours (Honig, 1968) and the CO_2 produced will replace the oxygen used up. Further production of fermentation gases will then occur, declining from 2500 l/100 kg dry matter at 15 per cent dry matter to 700 l/100 kg dry matter at 50 per cent DM. Dexter, (1966) obtained similar volumes of carbon dioxide from lucerne silages of different moisture content during the first 120 h of ensilage.

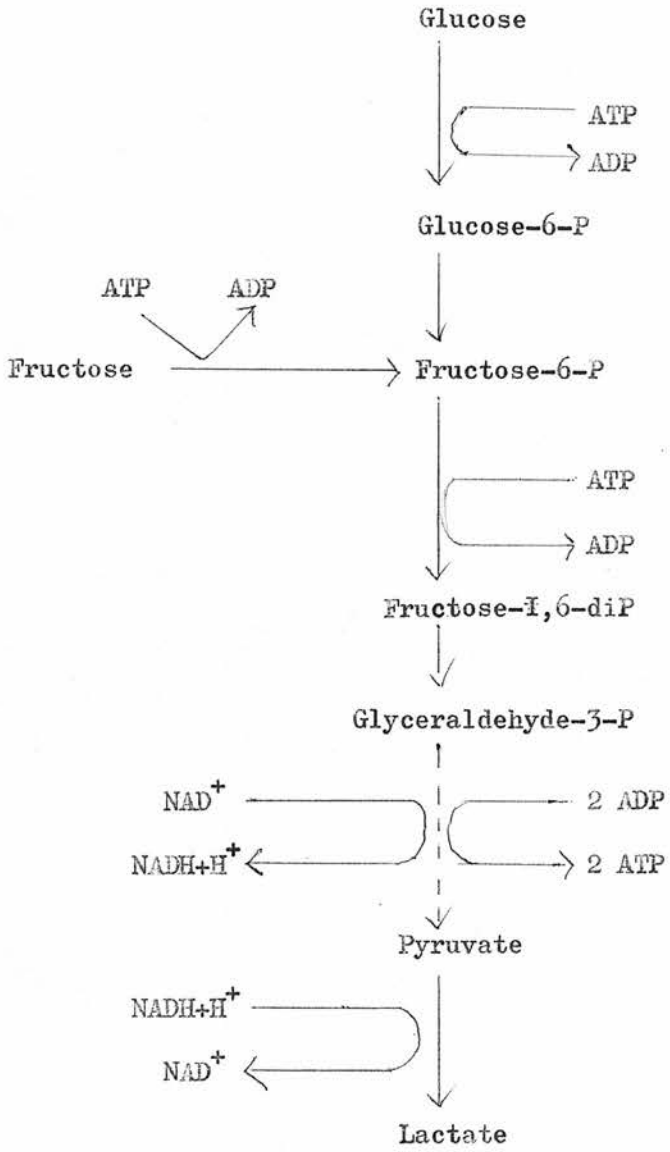
Fermentation by lactic acid bacteria will reach a maximum any time during the first fortnight, depending on environmental conditions. Under certain conditions lactate may be fermented by clostridia at a later stage. Yeasts may become dominant if the bacteria are inhibited.



Sum:- 2 Glucose + 3ADP $\longrightarrow$ 2 Lactic acod + Acetic acid + ethanol + 2formic acid + 3ATP

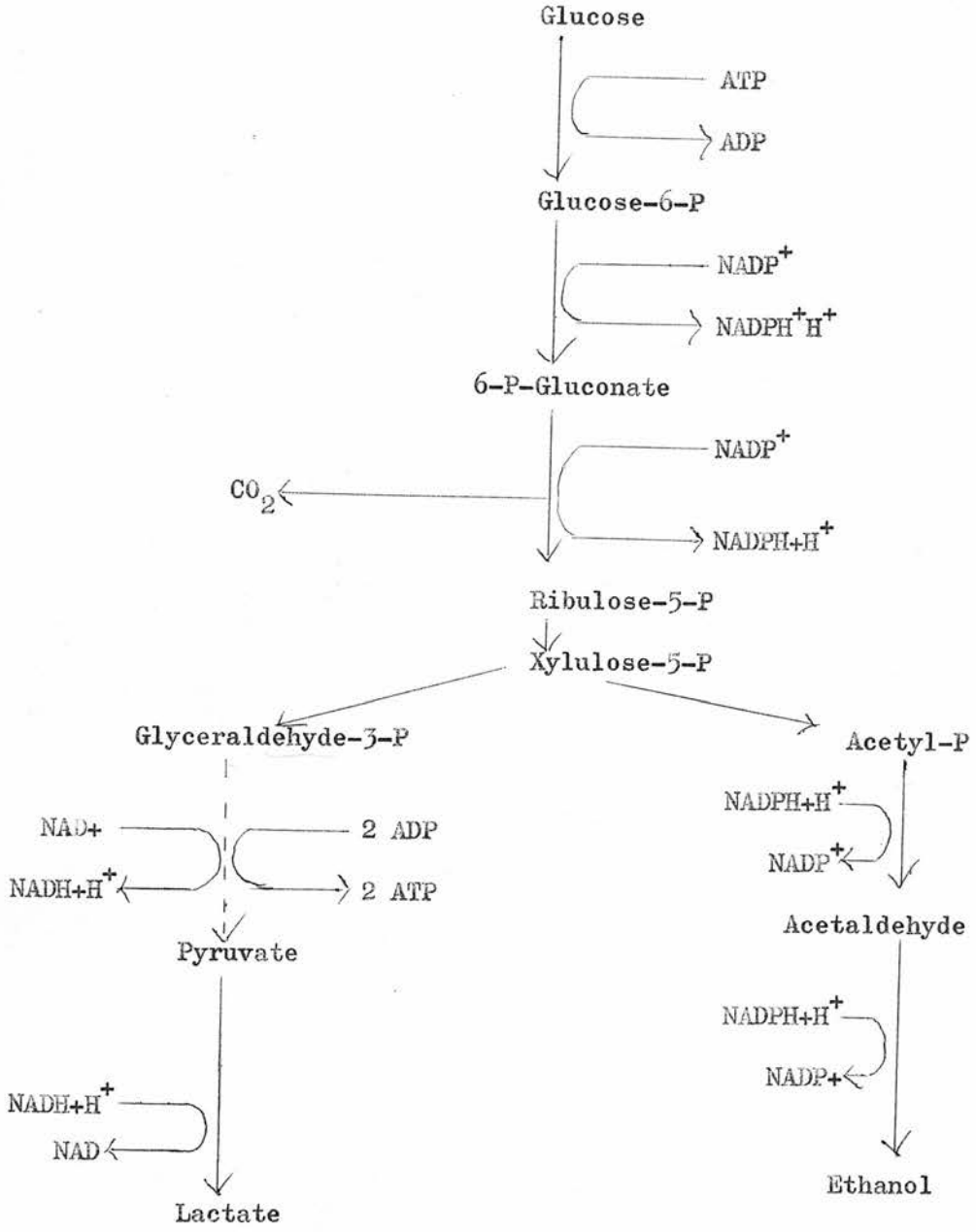
2 Formic acid $\longrightarrow$ $2H_2 + 2CO_2$

Fig I. Fermentation of glucose by Escherichia coli



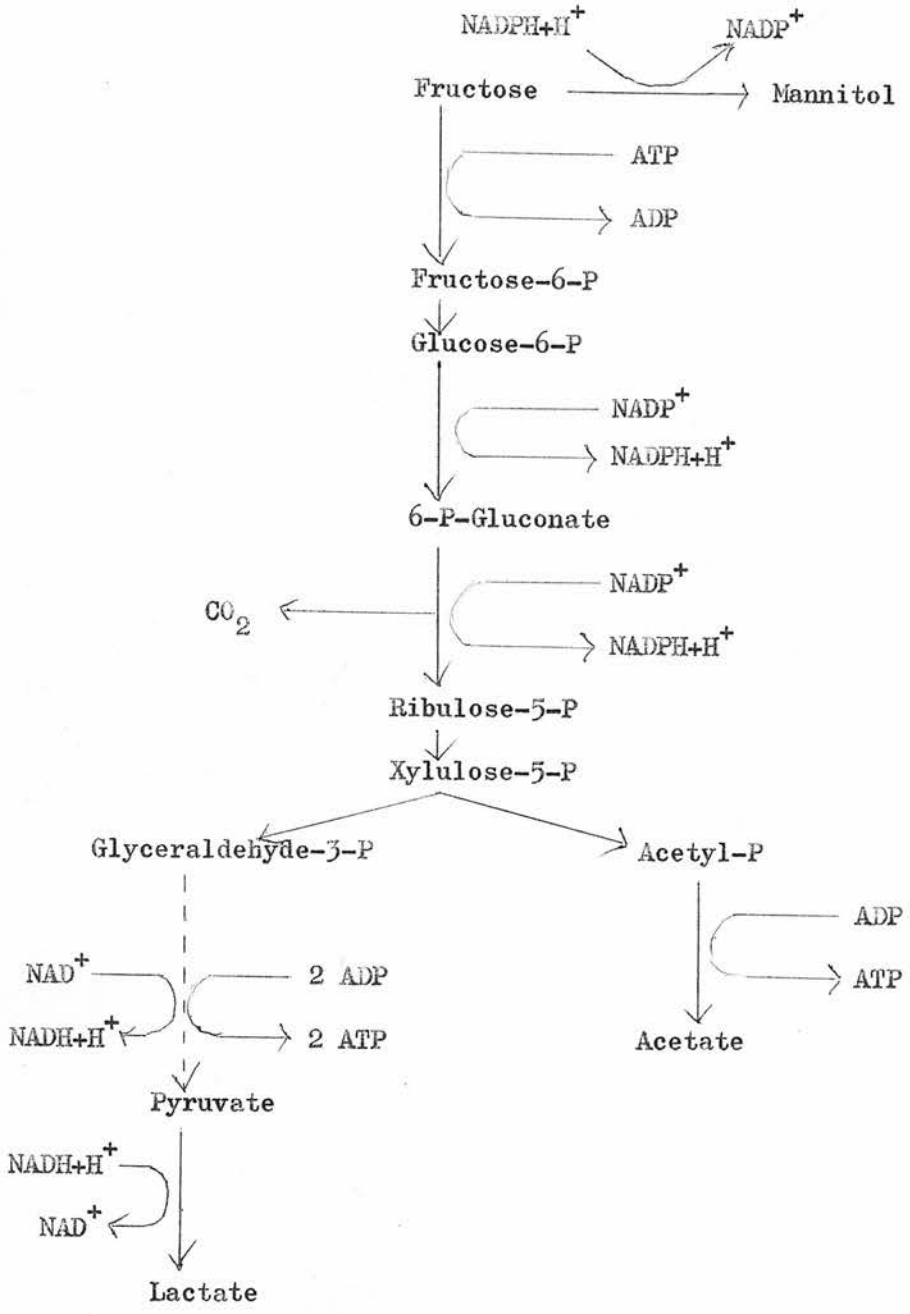
Sum:- $C_6H_{12}O_6$ + 2 ADP + 2 Pi ⟶ 2 $CH_3CHOHCOOH$ + 2 ATP

Fig II Homolactic fermentation of glucose and fructose.



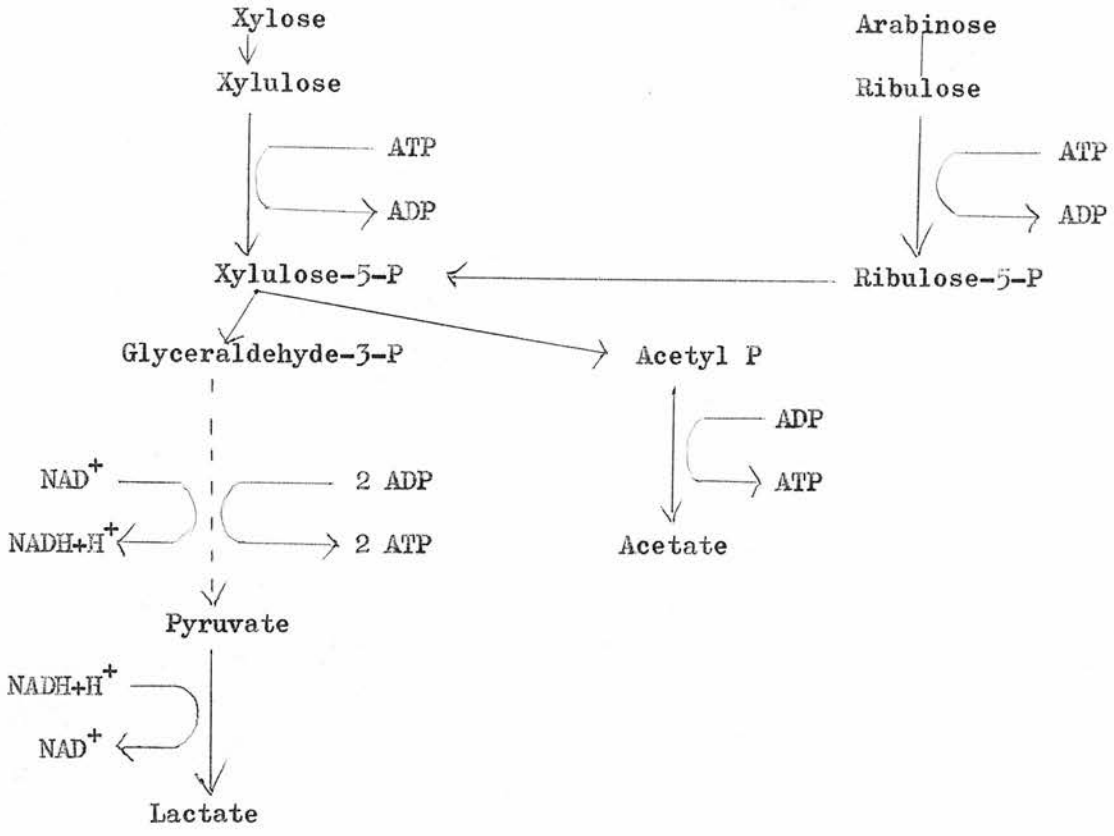
Sum:- $C_6H_{12}O_6 + ADP + Pi \longrightarrow CH_3CHOHCOOH + C_2H_5OH + CO_2 + ATP$

Fig. III Heterolactic fermentation of glucose.



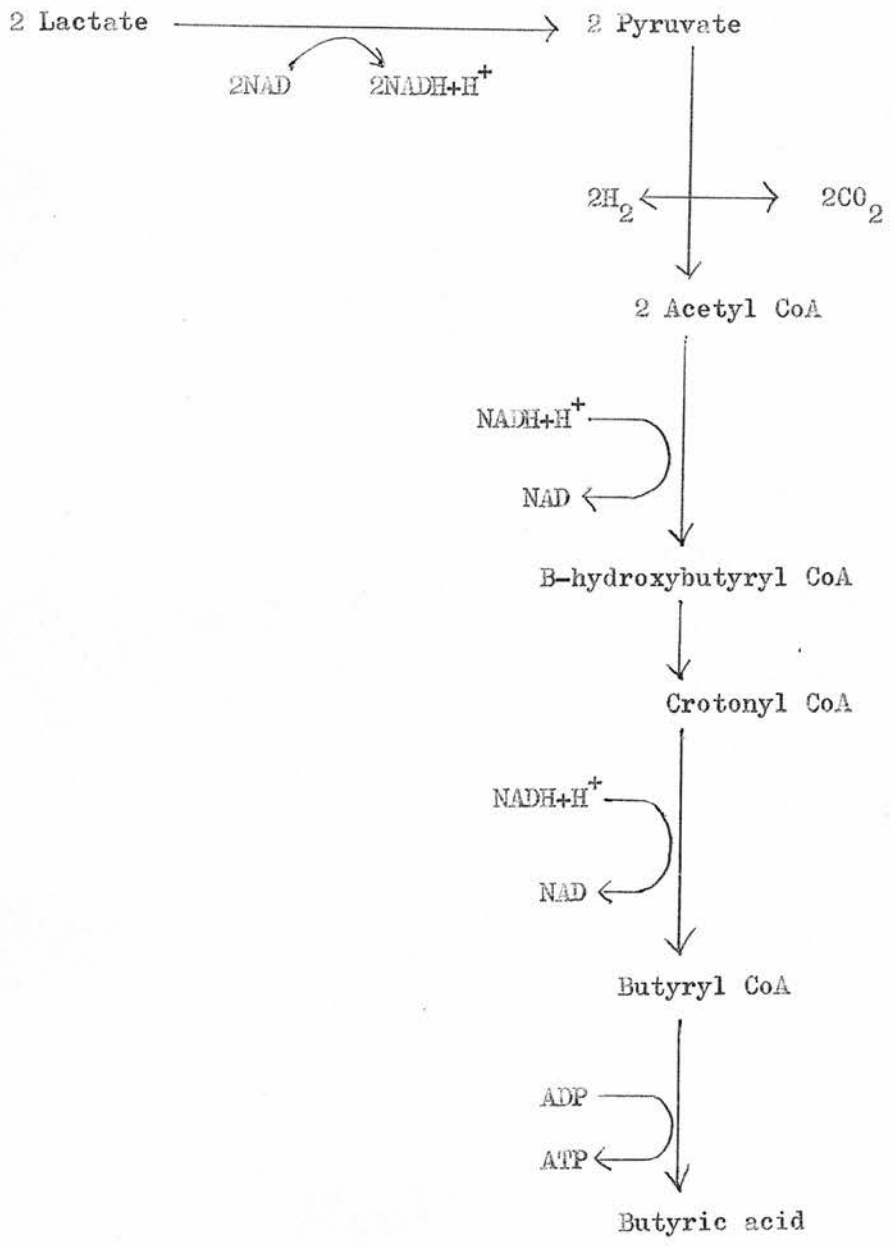
Sum:- $3\ C_6H_{12}O_6 + H_2O + 2\ ADP + 2\ Pi \longrightarrow CH_3CHOHCOOH + 2\ C_6H_{14}O_6 + CH_3COOH + CO_2 + 2\ ATP$

Fig. IV. Heterolactic fermentation of fructose.



Sum:- $C_5H_{10}O_5 + 2\ ADP + 2P_i \longrightarrow CH_3CHOHCOOH + CH_3COOH + 2\ ATP$

Fig V. Heterolactic and homolactic fermentation of xylose and arabinose.



Sum :- 2 Lactate + ADP ⟶ Butyric acid + $2CO_2$ + $2H_2$ + ATP

Fig. VI. Clostridial fermentation of glucose

II (3) THE EFFECT OF OXYGEN ON ENSILAGE.

The continued respiration of plant and microbial cells, resulting in the production of carbon dioxide and water at the expense of the available carbohydrates will continue in the silo until the oxygen is exhausted. The reaction is accompanied by a rise in temperature which in turn accelerates respiration. The effect of oxidation is thus two-fold, resulting in a loss of valuable carbohydrate and a rise in the temperature of the mass. Two areas of research have therefore been investigated, the effect of aeration and the effect of temperature on silage fermentation.

Early workers (Watson, 1939) advocated a rise in temperature of the herbage to avoid an undesirable fermentation. Murdoch (1960) stated that above 55°C a clostridial fermentation would not occur but the digestibility of the final product would be low and nutrient losses would be high due to the active aerobic respiration. Wieringa (1960) stored a number of silages at temperatures in the range of 20° - 60°C with 5°C intervals during the first week. The silage temperatures were reduced 5°C per week until all the silages were at 20°C. The optimum temperature for butyric acid production was found to be 35°C. Lanigan (1966) found that demonstrable differences between silages made in sealed silos, without preliminary respiration, at temperatures ranging from 25° to 45°C were generally small in comparison to respiration effects. Undesirable fermentation changes were generally maximal around 37°C. Watson and Nash (1960) stated that herbage submitted immediately to a maximum temperature is very different from that in which the temperature rise had been achieved by natural means. This would be accompanied by a change in chemical composition and therefore laboratory experiments in which silos are held at different temperatures do not necessarily simulate farm silos.

Greenhill (1964) found that in a well-packed and completely sealed silo entrapped oxygen was consumed within one hour and carbohydrate respired under such conditions was negligible. Wilson et al (1969) compared silages made in polythene containers with and without removal of air by evacuation and found them to be very similar in chemical composition. McDonald et al (1966) calculated that the initial volume of oxygen trapped in fresh grass (1000 kg) occupying a volume of $3.02m^3$ was $0.44m^3$ and this would oxidise 0.07 per cent glucose in the fresh herbage, raising the temperature by $2.8^{o}C$. Pedersen (1971) observed a temperature rise of $4^{o}C$ when he ensiled a grass/clover mixture in a sealed silo insulated with glass wool. The Australian workers (Lanigan et al (1966), using forced aeration of chopped ryegrass in containers thoroughly insulated against heat loss showed that as much as 8 per cent of the initial dry matter could be lost in raising the temperature to $45^{o}C$. In a low WSC crop a temperature rise of this magnitude could deplete the available carbohydrate to a level below that required to ensure satisfactory preservation of the material (McDonald et al 1966).

Langston et al (1958) found that the oxygen content of silages sealed immediately after filling dropped rapidly and no measurable amounts remained 5 hours after filling. Utilisation of oxygen in a silo sealed 48 h after filling was less rapid and oxygen was not detected at depths of more than 15 cm in an unsealed silo 14 and 39 days after storage. Poor chemical quality and distinctly lower rates of nutrient preservation characterised the untramped silages stored in unsealed silos. Within species, high storage temperatures were associated with poor silages. Lucerne was more tolerant of high temperatures than cocksfoot. The effect of extent of oxygen inclusion in the mass on the competitive ability of organisms during the early stages of ensilage was studied by Langston et al (1962). Aeration caused temperatures to rise markedly. Most reached a maximum within one day and then decreased gradually. The temperature

of the sealed silages did not increase by more than a few degrees. The aerated silages proved to be inferior in quality. Acid levels were higher in the sealed than in the aerated silages, indicating that some of the initial substrate available for fermentation was destroyed by aeration.

Differences in the volume of oxygen trapped in the silo will depend on the length of cut of the grass, compression, moisture content and speed of filling.

Watson and Nash (1960) summarised the effects on the losses during conservation of chopping the herbage and their data indicate a reduction of 3.5 per cent in the loss of dry matter. Murdoch et al (1955) confirmed that laceration resulted in a more rapid formation of lactic acid and a lower temperature rise during ensilage of the herbage.

McDonald et al (1966) demonstrated that the volume of moderately compacted fresh herbage fell more rapidly than that of wilted herbage treated in the same manner, but the temperature rise was more rapid in the fresh material, reaching a mean of 42°C on the third day. The effect on the fermentation of the aeration and temperature rise was greater in the fresh material than in the wilted. In each case the lactic acid content was lower in the aerated material. When Greenhill (1964) left two experimental silos containing ryegrass and lucerne open for 21 h the material gradually collapsed, the rate of collapse increasing after 2 - 3 h. When these two silos were sealed the oxygen was used up more slowly than in the silos sealed immediately. In the ryegrass silage the subsequent fermentation was as vigorous as in the control silage but in the lucerne silage the rate of acid production was much slower and it was thought that the continuous respiration may have depleted an already inadequate supply of fermentable carbohydrate.

Ohyama and Mosaki (1970) studied the effect of laceration and consolidation on ensilage in containers with mercury seals. The amount of organic acids was

generally less where the herbage was long and loosely compacted. The quality of the silage did not depend on the treatment, but, where the quantity of WSC in the ensiled herbage was limiting, the silages made from the long material were of poor quality.

Moulds will grow in the silo for a short time after filling but their growth will cease with the disappearance of oxygen. Exclusion of air from the silo will prevent incursion of moulds (Watson and Nash, 1960). Yeasts also occur in silage and their population density is said to be influenced by the level of oxygen present during the early stages of ensilage. (Weise, 1965; 1966 and Honig, 1969). Silages containing more than 10^5 yeasts per g showed a very poor stability when the silo was opened.

Most work has been carried out on the effect of oxygen during the early stages of ensilage but the effect of oxygen on the surface of a farm silo throughout the ensiling period can also be a major source of loss of nutrients. Using a marker technique (Wittwer et al, 1955), losses of up to 80 per cent of the stored dry matter can be measured in the spoiled layer, the highest losses occurring in the material nearest the surface, the lower losses occurring in the material adjacent to the good silage (Beltsville, 1959). This indicates that air infiltration is progressive and gaseous losses may account for a major portion of the losses due to exposure of the silage.

Daniel et al (1970) studied the effect of aeration at the rate of 30 ml and 150 ml of air per kg herbage (grass/clover) dry matter per day and found the effect of air to be slight and non-uniform. At the lower dry matter levels there was a definite decrease in lactic acid content but at the higher dry matter levels lactic acid content and level of air were only feebly correlated. In this experiment aeration during ensilage had only a slight effect on deterioration and production of carbon dioxide on exposure of the silage to the air.

In two experiments Honig (1969) aerated silos to a maximum value of 250 l of air per 100 kg herbage per day. During the later stages of ensilage CO_2 production was greater in the aerated silages. Production of acetic acid increased and lactic acid decreased with aeration. Butyric acid and alcohol content were unaffected although yeast counts increased with aeration. Changes occurred at the site of air intake, the longer the period of ensilage the further the damaged zone extended.

Pedersen (1971) ensiled (1) beet tops, (2) Lolium multiflorum and (3) a grass/clover mixture in experimental silos and supplied oxygen at increasing rates up to (1) 32, (2) 41 and (3) 60g O_2/100g DM to the silos as fast as it could be consumed. The maximum oxygen consumption per day in the beet top silage was about 0.5g per 100g DM and in the grass and grass/clover mixture about 3g/100g DM. When the silos were insulated there was a strong correlation between temperature in the silage and the oxygen supplied. Differences in appearance and smell were small among the silages supplied less than 5g O_2 per 100g DM. Above 15 - 20g O_2 per 100g DM the silages were worthless. Lactic acid content decreased and butyric acid content increased with increasing oxygen supply in the beet top silages but in the other silages the lactic acid content was almost independent of oxygen supply. There was no definite trend between oxygen supply and acetic acid content in any of the silages. The alcohol and WSC content decreased and the pH value increased with increasing oxygen supply in every case.

Gordon et al (1969) studied the influence of silo porosity on silage quality. Freshly chopped, first cut lucerne was ensiled in polythene bags (.025mm) and in glass jars. The silages from the polythene bags had high dry matter losses, low lactic acid content (0.8 per cent) and high acetic acid content (9.0 per cent). In comparison the silages made in the glass jars had low dry matter losses, high lactic acid contents (11.6per cent) and lower acetic acid contents (3.6 per cent).

II (4) FORMIC ACID AS AN ADDITIVE FOR SILAGE.

As the controlling factor in the natural ensilage process is the acidification of the mass, an acid, or an acid stimulant, would appear an obvious choice as an additive for silage. Virtanen (1947) studied the effect of mineral acids (A.I.V. acid) and found that reducing the pH value of the ensiled material to 3 - 4 was essential if the crop was to remain in a stable condition over a long period of time. Because of the danger of handling mineral acids and the possible danger to stock of feeding silages of this type, more emphasis is now placed on the use of organic acids, in particular formic acid.

Watson and Nash (1960) summarised the early work using organic acids to lower the pH value of the ensiled herbage and concluded that the process employing formic acid on the whole had not been successful. A comprehensive review of the use of formic acid as a silage additive up to 1969 was compiled by Easson (1970).

Pedersen et al (1968) reported on work carried out in Denmark to compare A.I.V. acid and formic acid when added to clover/grass mixtures. No significant differences were found in the effect of the two acids with regard to dry matter losses from the silo or quality of the silage. This was confirmed by Saue and Breirem (1969) using lacerated grass although extensive examination by the Norwegian workers of silage samples collected at farms gave varying results.

The introduction of the flail-type forage harvester and an applicator which could be attached to the forage harvester to spray concentrated formic acid on to the grass in the flail chamber (Aas and Naerland, 1966) influenced greatly the use and efficiency of this acid as a silage additive. Between the years 1956 and 1968 its use in Norway increased tenfold (Saue and Breirem, 1969). Since 1967 A.I.V. acid has not been manufactured in Norway. The use of formic acid in the U.K. increased when the proprietory product, ADD - F* (85 per cent formic acid) came on to the market in 1968.

* Manufactured by B.P. Chemicals International Ltd.

Experiments reported in the literature demonstrate that formic acid can affect the fermentation pattern in the silage in three ways. There are examples of silages in which formic acid has inhibited the lactic acid bacteria and produced a silage in which the lactic acid concentration is lower than in the control silage. When herbage low in WSC has been ensiled with the addition of formic acid, however, the lactic acid contents of the treated silages may be higher than those of the control silages, which are often of poor quality. Finally there are silages in which bacterial activity has been completely inhibited.

Examples of the first two types of treated silages were reported by Waldo et al (1971) although not all the silages complied with the above statements. In their 1967 experiments, silage made from lucerne of only 4.6 per cent WSC content in the dry matter had an unusually high lactic acid content (9.4 per cent) compared with the treated silage (5.7 per cent). In the three experiments conducted by Waldo et al (1969_a), using two varieties of cocksfoot and one of lucerne, 90 per cent formic acid was added at the 0.58 per cent, 0.53 per cent and 0.54 per cent level. WSC levels were not quoted for the ensiled herbage in this experiment but residual WSC contents were all low. The mean lactic acid content was 2.73 per cent for the control silage and 7.09 per cent for the treated silages. Volatile nitrogen as a percentage of the total nitrogen was reduced from 20.3 per cent to 7.0 per cent by the addition of the acid. Recovery of formic acid was equal to 41, 38 and 31 per cent of the acid added. Reasons given for the loss of formic acid were metabolism, volatilisation while filling, and seepage. The same workers claimed an increase in recovery of dry matter when formic acid was added to the crop (Waldo et al, 1969_b).

Wilkins and Wilson (1968) added 0.23 per cent formic acid to fresh S23 Lolium perenne containing 18 per cent WSC. The control and treated silages had the same pH value (3.8) but the control silage had a much higher lactic acid content (15.4 per cent) than the treated material (9.1 per cent). Neither

Neither silage contained butyric acid. Residual WSC contents were not given for these silages. The same concentration of formic acid was sufficient to produce a satisfactory fermentation in cocksfoot and lucerne of 12 per cent and 11 per cent WSC content respectively when the control silages were of poor quality but in their 1969 experiments, when the WSC contents of the cocksfoot and lucerne were 5 per cent and 7 per cent respectively, at least 0.69 per cent formic acid was required to produce a stable silage. The same workers (Wilson and Wilkins, 1972) added 0.25 per cent formic acid to autumn-sown rye, cut with shears and ensiled in test-tubes. In all cases the pH values of the treated silages were lower than the pH values of the untreated. In the opinion of these workers WSC content was not a limiting factor in this experiment and when the same material was forage-harvested satisfactory results were also obtained with the untreated material. This may have been due to inoculation by the forage harvester and more rapid release of plant juices or the slightly higher dry matter.

Wilson (1969_a) advocated the use of formic acid when ensiling autumn pastures high in clover and low in WSC content. When formic acid was added, the silage quality was improved from 'very poor' to 'excellent' and the production of butyric acid was inhibited. The same worker (Wilson, 1969b) found formic acid a disappointing additive when used on heavily fertilised spring grass. The loss of WSC in the effluent and the loss of dry matter were increased, lactic acid contents were lower and formic acid did not completely inhibit the production of butyric acid.

Castle and Watson (1970) noted a marked effect on the fermentation pattern with the addition of 0.23 per cent formic acid to timothy/ryegrass. The maximum temperature in the silo was lower by 10 - 15°C and the formic acid invariably produced a silage of lower pH and higher lactic acid content. Zelter (1960) found that formic acid was ineffective when added to fresh lucerne at the 0.2 per cent level.

Workers at the A.D.A.S. Experimental Husbandry Farms at Drayton, Greathouse and Liscombe have studied the effect of the addition of formic acid at the rate of 0.75 gallon/ton or 3l/ton (approx. 0.36 per cent) on both fresh and wilted material. When added to S23 Lolium perenne of 20.2 per cent dry matter and 13.5 per cent WSC content at Drayton (1971 Report) the maximum temperature in the silo was lower by 5°C. There was no difference in surface spoilage but fermentation losses and losses of dry matter in the effluent were slightly higher in the treated silages. In the previous two years, workers at Drayton found little advantage in treating grass with formic acid except for the lower temperature throughout the ensiling period and the slightly higher residual WSC content. When formic acid was added to wilted grass at both Liscombe and Drayton the treated material at the end of the ensiling period had a higher lactic acid content than the control.

Derbyshire and Gordon (1970) added 0.5 per cent formic acid to wilted cocksfoot (47 per cent dry matter) of 7.6 per cent WSC content and found very little difference in the silages. The treated material had a higher WSC content (5.6 per cent) and lower lactic acid content than the control. (WSC - 3.4 per cent; lactic acid - 3.6 per cent).

The possibility that formic acid may have a bactericidal effect was stated by Saue and Breirem (1969). They quoted Swiss workers who claimed in 1941 that formic acid had a selective bactericidal effect, undesirable silage bacteria being affected, but lactic acid bacteria hardly at all. Experiments carried out in Scandinavia on ensiling fish and other animal products also indicated that formic acid had a bactericidal effect. From their own work, Saue and Breirem noted that formic acid limited respiration and other undesirable fermentations during the early stages of ensilage. Temperatures remained much lower and production of carbon dioxide was reduced by half during the first days in the silo.

A decrease in the production of carbon dioxide has been reported by other workers using different acids (Watson and Nash, 1960; Daniel et al, 1970; Oestemer, 1972). Leroy and Zelter (1954) stated that formic acid, used at the 0.2 per cent level, inhibited the degradation of nutrients in the silo by the bacterial flora. Beck (1968) found that compounds containing formate at the lower range of concentrations stimulated the growth of clostridia, coliforms and yeasts.

Carpintero et al (1969) added formic acid at a level of 0.85 per cent to lucerne. This lowered the pH value to 4.2 immediately and from the absence of lactic and butyric acids in the silage it was obvious that this level of acid had been sufficient to inhibit the activity of lactic acid bacteria in the silo. Wilkins and Wilson (1969) found that it was necessary to add 1.5 gallons/ton (0.69 per cent) of ADD - F to cocksfoot or lucerne to obtain a stable silage of low pH value but that when 2 gallons/ton were added (0.92 per cent) the crop was sterilised.

III. Objects of study.

The object of this work was to study the water soluble carbohydrate fractions of grasses, the factors affecting them in the growing plant and their rôle in the ensilage process. As there may be a delay between the filling and the final sealing of a farm silo an experiment was designed to study the differences in losses and the products of fermentation of silos which were sealed immediately and of those after delayed sealing. A wide range of additives which may improve the quality of his silage is now available to the farmer. The aim was to study the effect of one of these, formic acid, on the water soluble carbohydrate fractions and on the course of fermentation in the silo of grass treated with this additive.

IV. TECHNIQUES.

IV. (1) SAMPLING.

In order to obtain a representative sample of grass from the standing crop, the field was walked in the pattern of the letter W and a handful of grass was cut at regular intervals with hand shears. The bulked sample was then carefully mixed and subsampled by 'quartering', i.e. by discarding half of the sample, remixing and discarding one half of the remainder. The subsample was cut into small lengths with hand shears in preparation for analysis. The results of the analysis of these samples when compared with the results of the analysis of the grass as ensiled gave an indication of soil contamination and changes in the water soluble carbohydrate (WSC) fraction between the field and the silo.

A sample of grass, representative of the material ensiled, was obtained as the silo was being filled by taking periodic hand samples which were stored in a large plastic bag. In a similar manner, hand samples of silage were taken as the silo was being emptied. The bulked samples, which represented about 1 - 2 per cent of the weight of the grass ensiled or silage removed, were thoroughly mixed, subsampled by 'quartering' and prepared for analysis as described above.

IV. (2) SILOS.

IV (2) 1. Metal Tower silos.

The silo unit, consisting of four metal tower silos used in experiments F1, F2 and F3, was the one described by McDonald and Atwood (1958). The silos, housed indoors, are constructed of mild steel and measure 153 cm in diameter by 183 cm in height with a total capacity of 3.02 m^3 or approximately 1 tonne of fresh grass. The base of the silo slopes gently to a central outlet pipe to allow free drainage of effluent. A plastic tube leads the effluent from the outlet pipe to a plastic container. Seven sampling ports

are arranged at different levels around one third of the circumference of the silo. The inside of the silo is graduated in cm so that the volume occupied by the herbage can be calculated at any given time. Ten thermocouples, connected to a single recording unit, lead into each silo and allow temperature readings to be taken at any time. Consolidation of the grass was obtained by tramping during the filling of the silos and finally by placing stone blocks on the plastic sheet covering the grass.

The silos are suspended from a weighing device of the steelyard type and the weight loss can be recorded at any time on a spring balance which is under tension. The steelyard permits a mechanical advantage of approximately 9 : 1. The true mechanical advantage was determined by counter-balancing known weights placed in the silo.

IV (2) 2. 'Plastolene' silos.

The 'bag' silos, capacity 2 - 3 tonnes, used in experiments 01, 02 and F6 were manufactured by Gordon Low (Prefabricators) Ltd., Isle of Wight, from a terylene mesh coated on both sides with polyvinyl chloride (P.V.C.). Brass taps were fitted to the bases of the silos for the removal of effluent and the silos were sealed by securing the hems between two lengths of rigid P.V.C. tubing, the outer tube split lengthwise to fit over the inner tube.

IV (2) 3. Test-tube silos.

The test-tube silos used in experiments F1 - F6 were Pyrex glass test-tubes, 20 cm in length and 2.8 cm in diameter with a capacity for approximately 80 g of fresh grass when tightly packed. The test-tubes were fitted with rubber stoppers carrying a valve of mercury over sintered glass which permitted the passage of gases but prevented the entry of air.

IV (2) 4. Laboratory tower silos.

The laboratory tower silos used in experiments F1 and F2, capacity ca. 1,200 g fresh grass, were constructed from glass tubing 42 cm in length and 10 cm in diameter and were fitted at both ends with a rubber stopper. The upper stopper carried a glass tube leading into an empty test-tube as a precaution against contamination from 'suck-back'. From the empty tube, the gases produced during ensilage were passed through a series of test-tubes containing a saturated solution of barium hydroxide which absorbed the carbon dioxide.

IV. (3) LOSSES.

Accurate losses during ensilage were calculated from the weight of fresh grass ensiled and the weights of fresh silage and effluent removed from the silo. When the metal tower silos were used these values were obtained *in situ*. The grass ensiled in and the silage removed from the 'Plastolene' silos were weighed in a trailer on a weighbridge. Effluent was weighed daily in a plastic container on a heavy duty balance. 'Waste' silage, i.e. inedible material was weighed separately. Losses of dry matter (DM) during ensilage were calculated by multiplying the weights of grass ensiled and silage and effluent removed by their true DM value. The total DM loss from the silo less the DM in the effluent is referred to as the 'fermentation and oxidation' loss. The 'edible' loss is the sum of the loss of DM in the waste material, the loss of DM in the effluent and the loss of DM due to fermentation and oxidation.

An estimation of the loss of grass DM involved in the production of waste silage was made by the 'bag and marker' technique of Wittwer *et al* (1958). This procedure was used in the metal tower silos only. When approximately three quarters of the grass was ensiled, the silo was weighed and a marker sheet of terylene net was placed over the herbage. Four polythene bags with

approximately 300 holes made in each with a cork borer were each filled with 4 kg of fresh grass and ensiled with the remainder of the grass above the marker sheet. The material in these bags was regarded as being representative of the 'good' silage above the net and the loss of DM from the bags as being equal to the loss of DM from the 'good' material. As the total weight of grass DM above the net was known, the weight of grass DM converted to waste silage was calculated by subtraction.

Terylene net bags containing a known weight of grass were ensiled in the 'Plastolene' silos to determine the loss of DM from the silos by the method described by Ruxton (1972). The material in the bags was regarded as being representative of the grass ensiled and silage removed and losses of DM were calculated after bulking and subsampling the silage from the bags.

IV. (4) ANALYSIS.

Details of some of the methods of analysis are given in Appendix 1.

IV (4) 1. Dry Matter.

The grass was dried in a forced air electric oven at 100°C for 24 hours and the dried samples were hammer milled through a 1.0 mm mesh sieve and stored in screw-capped bottles until analysed.

Silage DM values were determined by the toluene disillation method of Dewar and McDonald (1961). In addition, samples of silage were oven dried and milled in the manner described above and stored in screw-capped bottles until analysed. The results of analysis of the dried silage samples were corrected for the loss of volatiles by multiplying by a correction factor (oven DM ÷ toluene DM).

The DM content of the effluent was determined by the method of Dewar and McDonald. Where the ethanol content was high a correction was made for

this. In addition, an aliquot of the effluent was dried in an oven and a correction factor applied, as above, to the results of analysis of the dried material.

IV (4) 2. pH.

The pH value of the grass and silage was determined on a macerate of the fresh sample, using a Pye Ingold Electrode. The pH value of the effluent was determined on a fresh sample.

IV (4) 3. Buffering capacity.

The buffering capacity (bc) was determined on a macerate of the fresh grasses and silages as described by Playne and McDonald (1966) and was expressed as mg equivalents of alkali required to change the pH value of 100 g DM from 4 to 6.

IV (4) 4. Organic matter.

The organic matter (OM)was obtained by subtracting the ash content of the true DM from 100. The ash content of the dried, milled samples of grasses and silages and of the dried effluents was determined by a similar method to that described in the Regulations of the Fertilisers and Feeding Stuffs Act, 1955, using an electric muffle furnace.

IV (4) 5. Nitrogen.

Total nitrogen (TN) was determined in the dried, milled samples of grass and in fresh samples of silage and effluent by the normal Kjeldahl method. The crude protein (CP) contents of samples were obtained by multiplying TN by 6.25.

Total soluble nitrogen (TSN) or non-protein nitrogen (NPN) was determined in fresh samples of grasses and silages by extracting the samples twice with boiling water and determining TN in the combined extracts by a micro-Kjeldahl method.

Volatile nitrogen (VN) was determined in the NPN extract by steam distillation in the micro-Kjeldahl apparatus using 0.05 M sodium borate (pH ca 9.2) to liberate ammonia.

Nitrate nitrogen (nitrate - N) was determined on the dried, milled samples of grasses and silages and on aliquots of effluent by the method of ap Griffith and Johnston (1961).

IV (4) 6. Crude Fibre.

Crude fibre (CF) was determined in the dried, milled samples of grasses and silages by the method described in the Regulations of the Fertilisers and Feeding Stuffs Act, 1955.

IV (4) 7. Modified Acid detergent fibre.

Modified acid detergent fibre (MAD - F) was determined in the dried, milled samples of grasses and silages by the method of Van Soest (1963) modified by Clancy and Wilson (1966).

IV (4) 8. Cellulose.

Cellulose was determined in the dried, milled samples of grasses and silages by the method described by Crampton and Maynard (1938).

IV (4) 9. Lignin.

For the determination of lignin by the method of Ellis et al (1946) and modified by Waite (1964), small subsamples of chopped fresh grass or silage were spread on mesh-bottomed trays and dried rapidly over a fan heater. The samples were milled in a small hammer mill through a 0.8 mm sieve before analysis.

IV (4) 10. Water soluble carbohydrates.

The water soluble carbohydrates (WSC) of grasses and silages were determined in the filtrate of a macerate of fresh grass or silage by the method of McDonald

and Henderson (1964a). Aliquots of effluents were clarified by the addition of equimolecular volumes of cadmium sulphate and barium hydroxide at 90°C and the WSC were determined by the modified Somogyi method (Wiseman et al, 1960).

Individual WSC and mannitol were separated by a paper chromatographic technique using ethyl acetate: acetic acid: formic acid: water (18: 3: 1: 4) solvent system. Pentoses and hexoses were eluted from the paper strips and determined by a modification of the Somogyi method, fructans were determined by the Roe method (Arni and Percival, 1951) and oligosaccharides and sucrose were hydrolysed in 0.05N sulphuric acid before analysis by the Somogyi method. Mannitol was determined by the method of Kolthoff and Belcher (1957).

IV (4) 11. Organic acids.

The volatile fatty acids and succinic and lactic acids were determined in a 0.6N sulphuric acid extract of silage or in an aliquot of effluent by the silicic acid column chromatographic method of Lessard and McDonald (1966).

IV (4) 12. Ethanol.

Ethanol was determined in the filtrate of a macerate of fresh silage or in an aliquot of effluent by the method of Kozelka and Hine (1941) developed for the routine analysis of specimens for medicolegal purposes and modified by Kent-Jones and Taylor (1954).

IV. (5) BACTERIOLOGICAL INVESTIGATION.

The bacteriological populations of the fresh grasses and silages from the experimental silos were examined in small samples collected during the filling and emptying of the silos. The sampler wore sterilised gloves and the samples were stored in sterile bags. At no time were the samples allowed to come into contact with unsterile surfaces. In experiment F1, the development of bacteria

was studied at intervals in test-tube silages made under controlled conditions in the laboratory. The media used and the methods of examination were similar to those described by McDonald et al (1960). Tween acetate agar was prepared by mixing Laboratory Lemco, 0.5 per cent; peptone, 0.5 per cent; yeast extract, 0.5 per cent; Tween 80, 0.05 per cent; glucose, 0.5 per cent; agar, 1.5 per cent; tap water, 100 ml. Immediately before use, 10 ml. of 2 M acetate buffer were added to 100 ml of this medium. Yeasts and moulds were identified on malt extract agar.

V. THE INFLUENCE OF DELAYED SEALING ON CHEMICAL CHANGES DURING ENSILAGE.

V. (1) INTRODUCTION.

It has been shown from biochemical calculations that the losses of dry matter during the ensilage of grass in a sealed system should not exceed 4-6 per cent (McDonald and Whittenbury, 1967). In practice, losses of this order were found to occur when grass was ensiled in experimental silos, the herbage was well consolidated and the silos were sealed efficiently (Anderson and Jackson, 1970a and b; McDonald et al, 1960; Zimmer, 1971). When the silos were not sealed immediately, considerable aerobic respiration occurred and dry matter losses were much higher (McDonald et al, 1960). Although it is more difficult to measure the dry matter losses from a commercial silo, it is generally considered that these are well in excess of 4 - 6 per cent. Watson and Smith (1956) claimed that dry matter losses, including effluent and wastage, were of the order of 15 - 30 per cent. Losses of 50 per cent were not uncommon in stack and clamp silos due to overheating and wastage. Watson and Nash (1960) summarised the losses incurred in ensilage from 1938 onwards and stated that the ordinary silage process showed an average loss of 19.5 per cent of DM for 162 samples examined. Zimmer (1967) related dry matter losses to the type of silo used and DM content of the crop ensiled. The lowest dry matter losses were found in haylages ensiled in gas-tight silos and the highest losses (>40 per cent) in direct-cut material ensiled in stacks. There was a significant correlation between the airtightness of the silo and dry matter losses. Honig (1969) demonstrated that CO_2 production increased linearly with oxygen supply and the longer the period of ensilage the greater was the extent of the damage due to oxidation.

The main difference between ensilage on the experimental scale and on the farm scale is the time factor. In the laboratory, the silos may be filled and sealed within an hour or two of the grass being cut. On the farm, silos may be left uncovered overnight or over a period of days at the weekend or if weather conditions make further cutting impossible.

Two experiments were designed to examine the effect of delayed sealing on the chemical changes in the ensiled material, and to compare such changes with those occurring in material in silos which had been sealed immediately. In the first experiment, Lolium multiflorum, high in WSC content and low in CP content, was ensiled. In the second experiment, an autumn (third) cut of grass from the same field, low in WSC content and high in CP content, was ensiled.

V. (2) EXPERIMENTAL

The silos used in these experiments were the reinforced PVC bags of 2 - 3 tonne capacity, described earlier. In each experiment eight silos were filled, the grass in four being well consolidated and the silos sealed immediately. In each of the other four, the grass was built up on the base of the silo and left exposed to the air. Six terylene net bags, each containing 4 kg of fresh grass, were placed at random in the eight silos. After 72 hours (h), four of the eight 'silos' were unloaded, two of those which were sealed originally and two of those which had been left exposed to the air. The grass in each of the four silos was weighed and samples were taken from nine separate locations in each silo, three each from the top, middle, and bottom layers at 0, 60 cm and 120 cm from the outside. At the same time, the sides of the remaining two 'open' silos were rolled up and sealed. The silages were weighed and sampled in a similar fashion at the end of the ensiling period.

In experiment 01, the grass was pretreated with a compound fertiliser (N 23; P_2O_5 11; K_2O 11) applied at the rate of 377 kg/ha six weeks before cutting. The grass was cut at about 50 per cent ear emergence on 21 May 1971, with a flail-type forage harvester and ensiled. The weather was warm and sunny throughout the period of cutting. Gases were released from the sealed silos daily for the first five days. The silos were opened 72h (silos 5-8) and 80 days (silos 1-4) after being filled

In experiment 02, the grass ensiled was the third cut from the same field.

The grass was pretreated with a compound fertiliser at a rate equivalent to 100 kg N/ha on 6 August and was cut at about 70 per cent ear emergence on 20 August, 1971, with a flail-type forage harvester and ensiled. The weather was warm and sunny for several days prior to cutting and on the day of cutting. Gases were released from the 'sealed' silos daily for the first three days and the silos were opened 72h (silo 5-8) and 139 days (silo 1-4) after being filled.

In experiments 01 and 02, temperatures in two of the 'open' silos were recorded by eight thermocouples buried at different levels throughout the herbage. Two maximum thermometers were placed in each of the remaining six silos, one near the bottom, the other near the top. Ambient temperatures were recorded by two thermocouples during the first 72h of ensilage.

Effluent was removed daily from the 80 and 139 day (d) silages, weighed and the pH values recorded. Some effluent was lost from the sides of the silos sealed after 72h and, in the calculation of losses, this is included in the fermentation and oxidation loss.

V. (3) EXPERIMENT 01 - RESULTS.

The following fresh weights of grass were ensiled with the given treatments:-

A - Silo 1 - 1984kg - Sealed immediately and ensiled for 80d.

" - " 2 - 2110kg -Sealed immediately and ensiled for 80d.

B - " 3 - 1919kg - Open for 72h and sealed for 77d.

" - " 4 - 1811kg - Open for 72h and sealed for 77d.

C - " 5 - 2077kg - Sealed immediately and unloaded after 72h.

" - " 6 - 2018kg - Sealed immediately and unloaded after 72h.

D - " 7 - 1984kg - Open for 72h and unloaded.

" - " 8 - 2026kg - Open for 72h and unloaded.

V. (3) 1. Temperature changes.

Temperatures rose rapidly in the 'open' silos (7 and 8). At 20.00h on the day of filling, maximum temperatures of 30°C and 32°C were recorded near the

surface in these silos. The ambient temperature at that time was 20°C. After 24h, the maximum temperatures were 68°C and 69°C (ambient temperature - 14°C) and they rose to 71°C and 70°C after 72h (ambient temperature - 13°C). The recorded temperatures decreased with the increase in distance of the thermocouples from the surface layer. After 72h, the minimum temperatures (21°C and 38°C) were recorded near the bases of silo 7 and silo 8 respectively. The maximum temperatures recorded in the 'sealed' silos (5 and 6) opened after 72h were 21°C at the surface and 19°C at the base. The temperatures recorded on maximum thermometers taken from the top and bottom of the silos opened after 80d were:- silo 1 - 23°C, 21°C; silo 2 - 29°C, 25°C; silo 3 - 68°C, 27°C and silo 4 - 68°C and 31°C.

V. (3) 2. Composition.

The composition of the grasses ensiled in the eight silos is given in Table 1.

TABLE 1.

Composition of grasses.

Treatment	A		B		C		D	
Silo	1	2	3	4	5	6	7	8
DM (%)	21.9	22.4	22.5	22.4	20.7	21.1	20.8	22.3
pH	5.94	5.85	6.08	5.88	5.94	5.87	5.95	6.00
Bc (mequiv/100g DM)	26.5	-	-	-	-	-	-	-
Components of DM (%)								
OM	91.8	92.5	92.6	91.2	92.2	92.1	92.3	92.5
CP	10.6	10.4	10.7	10.2	10.7	10.8	11.2	10.6
CF	21.1	22.3	21.4	21.9	20.7	21.9	20.5	21.0
MAD-F	24.4	26.6	26.2	24.1	25.2	24.5	25.2	25.1
TN	1.70	1.66	1.71	1.63	1.71	1.73	1.79	1.70
PN	1.22	-	-	-	-	-	-	-
NPN	0.49	-	-	-	-	-	-	-

TABLE 1. (contd.)

Treatment	A				B		C	D
Silo	1	2	3	4	5	6	7	8
WSC	27.5	27.0	28.0	29.1	24.5	26.3	28.6	27.6
Glucose	5.0	4.8	5.1	3.4	-	-	-	-
Fructose	5.2	5.1	5.0	4.8	-	-	-	-
Fructans	11.4	11.4	11.7	12.3	-	-	-	-
Oligosaccharides (including sucrose)	4.3	4.1	4.5	7.2	-	-	-	-
Cellulose	23.1	23.2	23.2	23.2	23.4	23.4	23.0	22.7

The analyses of the grasses ensiled in the eight silos were similar with the exception of the WSC contents which ranged from 24.5 per cent of DM in silo 5 to 29.1 per cent of DM in silo 4. The ratio of fructose to glucose, after hydrolysis, in the grasses analysed for individual sugars was approximately 3 to 1.

After 72h the outer layer of the 'open' silos was green in colour but the inner core resembled good silage. The 'sealed' silos were uniform and still green in colour.

The composition of the nine samples of silage taken from the silos after 72h and 80d is given in Appendix 2 (Tables A1 - A5). The detailed analysis of the fermentation acids was only carried out on two samples from each silo (top 2 and bottom 2). A statistical analysis of the results detailed in Tables A1 - A4 is given in Tables 2a, 2b, 2c and 2d. In this, the outside samples (top 1, 2 and 3, middle 1, and bottom 1) were compared with the inside samples (middle 2 and 3 and bottom 2 and 3). All differences stated as significant are at the P = 0.05 level.

TABLE 2a.

Percentage DM.

Treatment	Total samples (18)	Position		SE of position differences
		Outside (10)	Inside (8)	
A	20.0	19.9	20.1	
B	19.2	18.1	20.4	±0.725
C	20.2	20.4	20.1	
D	19.8	19.8	19.7	
SE of treatment differences	± 1.040	±1.135	±1.186	
Marginal means	19.8 ± 0.368	19.5	20.1	
		SE of differences between marginal means ±0.362		

There was no significant difference amongst the DM contents of the samples with the exception of treatment B, in which the DM contents of the outside samples were lower than those of the inside samples.

TABLE 2b.

pH

Treatment	Total samples (18)	Position		SE of position differences
		Outside (10)	Inside (8)	
A	4.31	4.36	4.25	
B	4.33	4.41	4.24	±0.097
C	5.70	5.79	5.58	
D	5.23	5.75	4.59	
SE of treatment differences	±0.051	±0.079	±0.092	
Marginal means	4.89 ± 0.018	5.08	4.67	
		SE of differences between marginal means ± 0.049		

The pH values of samples from treatments A and B were significantly different from those from treatments C and D (A, B < C, D). After 72h the values of the inside samples from the 'sealed' silos were significantly higher than those from the 'open' silos and from the 'open' silos, the outside samples were significantly higher than the inside samples.

TABLE 2c.

Percentage WSC in DM.

Treatment	Total samples (18)	Position		SE of position differences
		Outside (10)	Inside (8)	
A	8.4	5.5	12.0	
B	7.9	2.4	14.7	±1.89
C	22.1	20.2	24.5	
D	18.3	17.2	19.6	
SE treatment differences	±1.11	±1.62	±1.85	
Marginal means	14.2 ± 0.392	11.3	17.7	
		SE of differences between marginal means ± 0.95		

The WSC contents of the final silages (A and B) were significantly lower than those of the silages opened after 72h (C and D) and those of treatment C were higher than those of treatment D. The WSC contents of the outside samples of treatment C were not significantly different from those of treatment D but the WSC contents of the inside samples from treatment C were significantly higher than those from treatment D. The WSC contents of the inside samples from treatment B were not significantly different from those of treatment D The WSC contents of the outside samples were significantly lower than the inside samples in treatments A, B and C. In treatment D there was no significant difference between inside and outside samples.

TABLE 2d.

Percentage MAD-F in DM.

Treatment	Total sample (18)	Position		SE of position differences
		Outside (10)	Inside (8)	
A	30.1	31.4	28.5	
B	34.2	37.4	30.1	±1.20
C	28.6	28.8	28.3	
D	30.8	31.7	29.7	
SE of treatment differences	±0.98	±1.24	±1.36	
Marginal means	30.9 ± 0.35	32.3	29.2	

SE of difference between marginal means ± 0.60

The MAD-F contents of the total samples from treatment B were significantly higher than those from the other treatments. This also applied to the samples from the outside but there was no significant difference among the inside samples. The MAD-F contents of the outside samples in treatments A and B were significantly higher than those of the inside samples but there were no significant differences between inside and outside samples in treatments C and D.

There was a higher concentration of organic acids in the samples from the bottom of the 'open' silos than in those from the 'sealed' silos after 72h. At this stage, succinic acid was the major acid in the 'sealed' silos but butyric acid was present in every sample analysed. At the end of the ensiling period, lactic acid contents were higher in the 'sealed' silages than in the 'open' silages. Acetic acid contents were low in all the samples, with the exception of the surface samples from the 'open' silages in which they were associated with low lactic acid contents.

Samples taken from silos 5, 6, 7 and 8 (middle 2) contained 0.9, 1.3, 1.0 and 0.8 per cent ethanol in the DM.

Chromatograms were run on water extracts of the nine silage samples taken from silos 5, 6, 7 and 8 and these were examined qualitatively for glucose, fructose, xylose, galactose, arabinose and mannitol. The results given in Table 3 were obtained from a visual evaluation of the relative density of the chromatographic spots.

TABLE 3.

Individual sugars.

Treatment	Silo	Sample		Glucose	Fructose	Galactose	Mannitol
C	5	Top	1	xx	xx	nil	nil
			2	xx	xxx	nil	tr.
			3	xx	xxx	nil	nil
		Middle	1	xxx	xxx	nil	tr.
			2	xx	xxx	nil	x
			3	xx	xxx	nil	tr.
		Bottom	1	xxx	xxx	tr.	x
			2	xxx	xxx	tr.	x
			3	xxx	xxx	tr.	x
C	6	Top	1	xxx	xxx	tr.	nil
			2	xxx	xxx	nil	nil
			3	xx	xx	nil	nil
		Middle	1	x	xx	nil	tr.
			2	xxx	xxx	nil	tr
			3	xx	xxx	tr.	x
		Bottom	1	xxx	xxx	tr.	tr.
			2	xxx	xxx	tr.	tr.

TABLE 3. (contd.)

Treatment	Silo	Sample		Glucose	Fructose	Galactose	Mannitol
C	6	Bottom	3	xxx	xxx	nil	nil
D	7	Top	1	xxx	xxx	nil	tr.
			2	xx	xx	nil	nil
			3	x	x	nil	nil
		Middle	1	xxx	xxx	tr.	nil
			2	xx	xxx	tr.	x
			3	xx	xxx	x	tr.
		Bottom	1	xx	xxx	tr.	xx
			2	xx	xxx	x	x
			3	xx	xxx	nil	x
D	8	Top	1	xx	xxx	nil	tr.
			2	xx	xxx	nil	nil
			3	xxxx	xxxx	nil	nil
		Middle	1	xxx	xxx	tr.	nil
			2	xxx	xxx	tr.	tr.
			3	xxx	xxx	x	tr.
		Bottom	1	xx	xx	x	xx
			2	xxx	xxx	tr.	xx
			3	xx	xxx	tr.	xx

Xylose and arabinose were not visible on the chromatograms. The highest concentrations of galactose and mannitol were seen on the chromatograms run on extracts of inside samples from silos 7 and 8.

In addition to the nine samples from each silo, bulked samples were taken from the silages opened after 80d and the composition of these is given in Table 4.

The 'open' silos had an outer layer of dark material containing small patches of mould. The inner core was very light in colour but darkened on exposure to air. The 'sealed' silages were uniform, resembling a good silage. The over-heated outer layers (O) from silos 7 and 8 were isolated from the inner cores (I) and analysed seperately. The analysis of the effluents is given in Table 5 and the variations in pH value of the effluents from silos 1 and 3 are shown in Figure 1. The pH values of the effluents from silos 2 and 4 followed the same patterns as those from silos 1 and 3 respectively.

TABLE 4.

Composition of silages.

Treatment	A		B			
Silo	1	2	3		4	
			O	I	O	I
DM (%)	20.1	20.4	22.4	21.2	20.0	20.7
pH	4.28	4.30	4.72	4.49	4.76	4.28
Bc (mequiv/100g DM)	99	106	82	90	83	81
Components of DM (%)						
OM	90.6	87.6	88.6	91.4	89.4	92.1
CP	11.3	11.2	12.9	11.6	13.1	10.8
CF	25.5	25.0	27.2	25.5	29.8	24.9
TN	1.81	1.79	2.06	1.85	2.09	1.72
PN	0.52	0.64	1.21	0.85	1.19	0.75
NPN	1.29	1.15	0.85	1.00	0.90	0.97
VN	0.21	0.18	0.17	0.14	0.18	0.15
VN as % TN	11.5	9.9	8.3	7.6	8.6	8.7
WSC	8.7	9.7	11.1	14.0	10.3	14.4
Glucose	0.3	0.4	-[a]	1.3	-	1.6

TABLE 4. (contd.)

Treatment	A		B			
Silo	1	2	3		4	
			O	I	O	I
Fructose	6.2	7.5	-	5.3	-	6.5
Xylose	0.5	0.6	-	0.1	-	0.1
Galactose	0.3	0.2	-	nil[b]	-	0.1
Arabinose	0.3	0.2	-	0.1	-	0.1
Oligosaccharides (including sucrose)	0.9	0.3	-	1.6	-	1.0
Fructans	0.3	0.5	-	5.0	-	4.4
Mannitol	7.7	6.6	-	3.7	-	4.0
Cellulose	28.1	28.2	29.7	27.1	31.9	27.9
MAD-F	31.6	33.9	34.0	30.7	35.0	29.4
Acetic acid	1.0	0.8	2.5	1.0	2.6	1.7
Propionic acid	0.4	0.2	0.7	tr.[c]	0.2	tr.
Butyric acid	2.0	2.7	3.5	1.3	4.7	1.4
Lactic acid	8.8	5.3	3.4	3.8	3.2	8.0
Succinic acid	2.3	1.7	0.2	0.4	0.3	1.6
Ethanol	2.0	2.0	0.6	1.6	0.6	1.3

a) - = not determined

b) nil = not present

c) tr. = trace.

The silages from the four silos were well-preserved, although the outer layers from the 'open' silos had relatively high pH values. The residual sugars were high in every silage but especially in the 'open' silages which had surprisingly high fructan contents. Mannitol contents were lower in these silages. Acetic

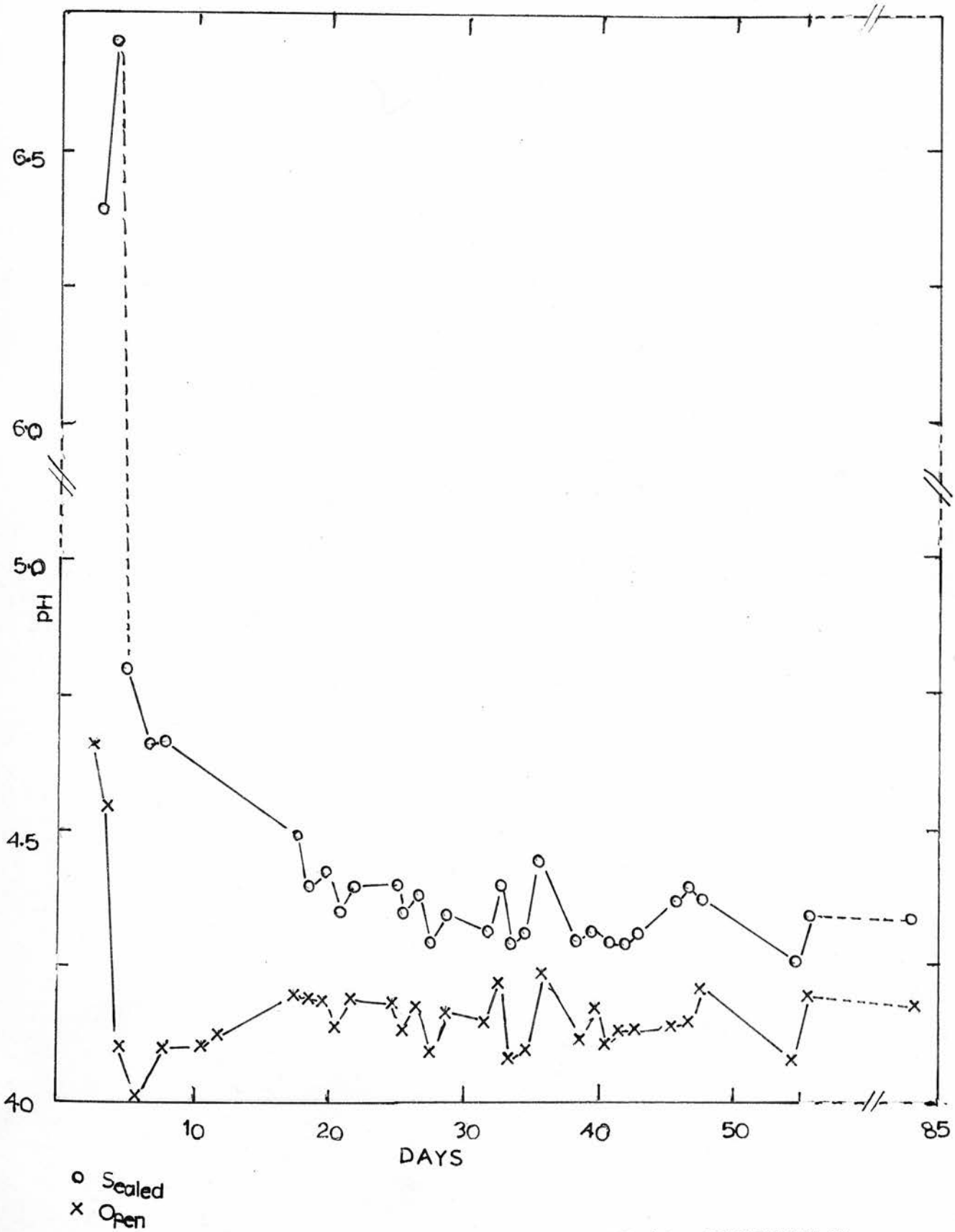


Fig. 1. Changes in effluent pH with time EXPERIMENT 01.

acid contents were lower and succinic acid contents higher in the 'sealed' silages. Lactic acid levels were lowest in the outer layers of the 'open' silos. For well-preserved silages, all contained higher than normal quantities of butyric acid. Proteolysis was greater in the 'sealed' silages than in the 'open' silages and volatile-N contents were slightly higher in the 'sealed' silages than in the 'open' silages but they were low in both treatments. The buffering capacities of the 'sealed' silages were higher than those of the 'open' silages. The MAD-F contents of the outer layers of the 'open' silages were high. The high fructose/glucose ratio in the grasses was reflected in the high mannitol contents of the silages.

TABLE 5.

Composition of effluents.

Treatments	A		B	
Silo	1	2	3	4
DM (%)	8.0	7.9	8.7	8.6
Components of DM (%)				
OM	82.1	81.7	83.2	84.4
N	3.1	3.2	2.8	2.5
WSC	6.4	6.8	11.8	16.7
Mannitol	19.9	23.0	21.0	24.0
Ethanol	19.0	13.0	13.5	14.3

Because of the high ethanol contents of the effluents, a correction was made for ethanol loss to the figure obtained for DM content determined by the toluene-distillation method. The lower NPN and higher WSC contents of the silages from silos 3 and 4 than those from Silos 1 and 2 are repeated in the ~~lower~~ values obtained for these constituents in the effluents from silos 3 and 4 and in the effluents from silos 1 and 2. The pH values of the effluents from the 'sealed' silos fell slowly and did not reach pH

4.3 until the 28th day, thereafter remaining fairly constant. The pH values of the effluents from the 'open' silages fell rapidly to pH 4.0 on the sixth day but rose again until they reached pH 4.15 on the 18th day, after which they remained steady.

V. (3) 3. Microbiological assay.

The results for microbiol counts on the standing crop and the grasses as ensiled are given in Table 6, together with counts on the flails of the forage harvester in the morning and in the afternoon. The results of the samples taken at nine positions throughout the silos are given in Appendix 2 (Table A6).

TABLE 6.

Microbiological assay of grasses.
(counts/10g fresh grass)

Sample		VRBA*	TAA	MEA	YEA	LF	PC
Standing crop		2.1×10^4	1.0×10^2	3.2×10^2	1.3×10^7	<10	<10
Grass in silo	1	2.4×10^5	4.3×10^2	1.0×10^3	1.5×10^8	$>10^3$	$>10^3$
	2	-	-	-	-	-	-
	3	5.4×10^4	9.0×10	$2,5\times10^2$	7.8×10^7	<10	$>10^3$
	4	1.4×10^5	1.5×10^2	2.0×10^2	7.1×10^7	$>10^2<10^3$	$>10^3$
	5	6.6×10^4	7.0×10	7.4×10^4	3.9×10^7	<10	$>10^3$
	6	4.6×10^5	4.0×10^2	2.9×10^4	6.0×10^7	$>10<10^2$	$>10^3$
	7	5.1×10^5	4.7×10^6	7.7×10^4	7.2×10^7	<10	$>10^3$
	8	1.4×10^5	4.2×10^2	9.0×10^2	5.2×10^7	$>10<10^2$	$>10^2<10^3$
Flail in the morning (08.00h)		3.5×10^5	3.2×10^2	6.3×10^3	7.9×10^7	$>10^2<10^3$	$>10^3$
Flail in the afternoon (14.00h)		2.4×10^5	2.4×10^6	4.0×10^4	7.6×10^7	$>10^3$	$>10^3$

* VRBA - Violet red bile agar (Coliforms)

TAA - Tween acetate agar (Lactic acid bacteria)

* MEA - Malt extract agar (Yeasts and moulds)

YEA - Yeast extract agar (All organisms)

LF - Lactate fermenters

PC - Proteolytic clostridia

Counts of lactic acid bacteria were low on the standing crop but increased after the crop was harvested. Counts of coliforms, yeasts and moulds, and proteolytic clostridia were high on the grasses as ensiled. The counts of coliforms on the herbage after 72h were not measured in this experiment but the yeast and mould counts were still high. At the end of the ensiling period coliform and yeast and mould counts were low.

V. (3) 4. Losses.

The weights (kg) of fresh silage and effluent removed from the silos were:-

Treatment	Silo	Silage		Effluent
		Outside	Inside	
A	1	nil	1765	70
	2	nil	1877	74
B	3	455	1143	81
	4	350	1169	90
C	5	nil	2036	-
	6	nil	2019	-
D	7	nil	1769	-
	8	nil	1862	-

Detailed DM losses are given in Table 7 and losses of some components of DM are given in Table 8.

TABLE 7.

Percentage DM losses during ensilage.

Treatment	A		B		C		D	
Silo	1	2	3	4	5	6	7	8
Fermentation and oxidation	17.3	17.5	18.8	21.1	-	-	-	-
Effluent	1.2	1.2	1.6	1.9	-	-	-	-
Total	18.5	18.7	20.4	23.0	4.4	7.5	15.4	14.7
DM losses calculated from terylene bags	11.9	19.7	15.7	15.6	2.6	5.7	8.5	3.1

Effluent DM losses were low from the silages after 80d but total losses were high due to the exceptionally high fermentation and oxidation losses from the silages.

TABLE 8.

Percentage losses of DM components from 80d silages.

Treatment	A				B			
Silo	1		2		3		4	
	Silage	Effluent	Silage	Effluent	Silage	Effluent	Silage	Effluent
OM	19.6	1.2	23.0	1.1	22.1	1.3	22.8	1.8
WSC	74.3	0.3	70.8	0.3	62.6	0.7	64.4	1.1
MAD-F	+5.5	-	+3.6	-	3.7	-	2.1	-
Cellulose	0.9	-	1.3	-	4.4	-	4.5	-

Losses of WSC were higher from the 'sealed' silages than from the 'open' silages.

V (4) DISCUSSION.

It is generally accepted that when grass is ensiled in a sealed system the entrapped oxygen is rapidly consumed and bacteria capable of anaerobic growth multiply immediately with a resultant rapid fall in pH value (McDonald et al, 1966a; Nilsson, 1956). The high pH values obtained from the sealed silages examined after 72h are therefore surprising. It would appear that bacteria had been inhibited in some way e.g. by low initial counts, low ambient temperatures or lack of nutrients. McDonald et al (1960) ensiled an autumn cut of Lolium multiflorum from which laboratory silos were filled. The pH value of the effluent from the experimental tower silos fell slowly to pH 4.3 but the fact that the pH value of the laboratory silages, incubated at 30°C never fell below pH 5.1 was explained by the negligible counts of lactobacilli on the grass. Gouet et al (1970) ensiling Medicago sativa in laboratory silos found that the lactic acid bacteria developed slowly after a latent phase of three days. In the experiment reported here, counts of lactic acid bacteria were low on the standing crop but these increased on the cut crop.

Wieringa (1959) found that bacteria grow more slowly at 20°C than at 35°C and this has been confirmed by other workers (Nilsson, 1956; Lanigan, 1963; Stirling, 1951). The ambient temperature on the day of filling was 19.5°C but the temperature fell to 14°C after 24h and this may have influenced the fermentation in the 'sealed' silages. A maximum temperature of 22°C was recorded on a thermometer buried near the surface of the herbage. Gibson et al (1958) found that the decrease in the pH value was most rapid in silages held at 40°C and slowest in those held at 22°C but that the pH curves tended to approach each other with time. The low temperature rise in a sealed system is in accord with the calculations of McDonald et al (1966a) and with the observations of Langston et al (1962)

Another possible explanation for the apparent inhibition of the bacteria is nutrient deficiency. As there was an adequate supply of WSC, the substrate in short supply could have been in the amino acids fraction. Brady (1966) claimed that lactobacilli and pediococci require a range of amino acids for growth. In each of the four seasons in which he examined the nitrogen extract of leafy immature ryegrass, he noted the low quality of its nitrogen substrate and claimed that it was inadequate to allow high acid production. Although there appears to be nothing abnormal about the microbial counts after 72h the multiplication phase is commonly completed before much acid has accumulated (Gibson et al, 1958). When Sprague and Taylor (1965) ensiled Dactylis glomerata in PVC bags the pH values of forages grown at low fertility changed more slowly than those grown on fertile soil, the former fell below pH 4 after 125h, the latter after 18h.

It is interesting to note that the early accumulation of gas in the sealed silos was not affected, either by the low temperatures or the retarded activity of the lactic acid-producing bacteria. This gas, released from the sealed silos daily for the first five days, may have been produced by bacteria of the coliform group which yield a considerable variety of endproducts including carbon dioxide, hydrogen, formic acid, acetic acid, lactic acid, succinic acid, ethanol and 2, 3 butane-diol. (Stanier et al, 1963). Another source of the gas may have been anaerobic respiration which can yield pyruvic acid, lactic acid, acetaldehyde, ethanol and CO_2. (Barnett, 1954; Dexter, 1966; Honig, 1968). The counts for yeasts and moulds on malt extract agar were high for the grass samples and the values obtained for ethanol content of the samples taken from the centre of the 'open' and 'sealed' silos after 72h suggest that the gas could also have been a product of yeast activity, apparently unaffected by temperature rise.

In the 'open' silos the maximum temperatures were recorded by thermocouples near the surface. This is in agreement with the work of Langston et al, (1958). These workers stated that measurable amounts of oxygen were not found at depths

of more than 6" (25cm) in the central part of an unsealed silo 14d after filling. As the surface silage was of relatively low density, the explanation offered was that the biological activity of the 25cm layer was sufficient to reduce the oxygen as it infiltrated the surface. In the same way, the very high temperatures near the surface of the 'open' silos during the first 72h suggest that the loss of WSC by oxidation occured in the outer layers of the herbage only. The exothermic reaction was sufficient to raise the temperature in the main mass of the herbage and possibly to stimulate the bacterial activity. The different temperatures in the 'open' and 'sealed' silos did not appear to affect the types of bacteria present, only the rate at which they converted the WSC to acids. After 72h all silages contained some butyric acid and, after 80d, appreciable amounts of this acid were present. This could have resulted from the direct action of saccharolytic clostridia which attack sugars (Barnett, 1954), as the butyric acid appeared at the initial stages and not, as is more usual, as the result of a secondary fermentation. Gibson (1965) stated that certain clostridia were known to develop and multiply as early as do other silage bacteria. The very high DM losses are typical of those obtained from silages in which a clostridial fermentation has occurred (McDonald and Whittenbury, 1967). The high butyric acid contents are unusual in good quality silages of low pH value but Anderson and Jackson (1970a) produced silages, made from grass of 13.0 per cent CP in the DM, which had a pH value of 4.03 and contained 1.67 per cent butyric acid in the DM with a DM loss of only 4.7 per cent and Langston et al (1962) reported values of 0.45 per cent butyric acid at pH 3.8. When Archibald (1953) used sulphur dioxide as a preservative in the ensilage of a grass/legume mixture, there was an increase in the WSC content during ensilage, the pH value of the silage was 4.4, and the butyric acid content was high. As the value for acetic acid was very low and the value for lactic acid was normal, Archibald concluded that the fermentation pattern was different from that which usually occurred in the ensiling process.

The slow decrease in pH value in the 'sealed' silos would prolong the life of the coliforms and other bacteria found on the grass and would result in the loss of nutrients due to the activity of these bacteria. Zimmer (1971) claimed that only 2-3 per cent WSC in the fresh matter was necessary to obtain a satisfactory fermentation during ensilage. A higher WSC content led to an increase in yeast fermentation and therefore an increase in DM loss. Although the ethanol contents of the 'open' and 'sealed' silages were not exceptionally high , yeast activity could have contributed to the high DM losses.

The high levels of residual WSC in these silages after 80d were remarkable. In previous work with grasses of high WSC content, most of the WSC was used up during the ensiling period, unless the bacteria were inhibited by the addition of an additive (e.g. formic acid) or by wilting (McDonald et al, 1968). Weise (1965) found that, in the production of raw potato silage, less acid was formed with high bacterial counts than with low bacterial counts. Low external temperatures retarded the initial development of the organisms and therefore had a favourable effect on the course of the fermentation. Stirling (1951) incubated grass at 22°C and 30°C and found that the lower temperature led to a rise in the viable count up to ten days as against a rise to a peak at two to three days at 30°C. Maximum counts were higher at the lower temperatures. Although the fermentation in the 'sealed' silos was retarded in experiment 01, on average the lactic acid contents were higher and the WSC contents were lower at the end of the experiment, suggesting that the fermentation had been more efficient. In an earlier experiment in which partially wilted grass was ensiled (McDonald et al, 1966), the low temperature silage contained more lactic acid and less WSC than the high temperature silage.

Little is known about the breakdown of fructans to fructose during ensilage. Lanigan (1966) stated that none of the common strains of silage bacteria utilise ryegrass fructans and the acid hydrolysis of fructans at pH 4.0 would be much

slower than the actual rate of breakdown normally found in silage. He assumed that the reaction must be enzymic and produced a CP preparation from flowering ryegrass which possessed the appropriate hydrolytic activity. The low CP content of the ryegrass ensiled in this experiment may have been the limiting factor and may explain the high residual fructan content of the 'open' silages. The low level of fructan in the 'sealed' silages is then more difficult to explain unless the enzymic hydrolysis is more efficient at lower temperatures. Although the grass ensiled by Anderson and Jackson (1970a) had a low fructan content (4.46 per cent in DM), these silages were relatively high in fructan content (1.06 per cent in DM). The higher levels of protein N in the 'open' silages suggest that proteolysis is also more extensive at lower temperatures.

The values obtained for succinic acid in the 'sealed' silages are higher than those found in many silages. Kempton and San Clemente (1959) stated that well-preserved silages contain a small constant level of succinic acid. The high levels in the 'sealed' silages suggest that succinic acid may be associated in some way with a slow fermentation. Ruxton (1972) obtained high values for succinic acid with high inclusion of air in the silo initially. Again the rate of fermentation was slow. The acid was thought to be produced from the enzymic or bacterial breakdown of malate (Lessard, 1966) but is also a product of the coliform bacteria (Stanier, *et al*, 1963).

As would be expected, the losses of DM from the silages open for 72h were higher than those from the silages sealed for the same period, but these differences cannot be attributed entirely to oxidation losses as the fermentation had progressed in the 'open' silages. Losses of effluent DM were low from all the silages. The outer layers of silage from silos 3 and 4 could not be termed spoilage and would have been acceptable to stock. The values obtained for MAD-F content demonstrate clearly that the oxidation losses were concentrated on the surface layers of silos 3, 4, 7 and 8. The recoveries of MAD-F and cellulose obtained in this experiment

suggest that, if the MAD-F and cellulose contents of the ensiled grass are known, their contents in a farm silage could be used as a measure of losses with a $\pm$ 5 per cent accuracy. The disparity between the results from the input/output balance and the terylene net bags is thought to lie in the limited number of bags used and the consequent inadequacy of their distribution (Ruxton, 1972).

This experiment confirmed the work of Murdoch (1960) and Harris and Raymond (1963) that grass silages may be allowed to heat up over a short period of time with no deleterious effect on the silage quality when the crop ensiled is likely to support a desirable fermentation i.e. when the initial WSC content is high. Losses of DM during ensilage were not quoted by these workers. In the experiment reported here, oxidation losses were low in comparison with fermentation losses. This may have been the indirect result of ensiling a crop of low CP content and high WSC content but results from other work suggest that there must have been another factor influencing the fermentation pattern. In an experiment using Lolium multiflorum of low CP content (11.22 per cent in DM) and high WSC content (21.17 per cent in DM), Anderson and Jackson (1970b) produced a silage of low pH value (3.90) containing very little butyric acid (0.08 per cent in DM) and with a DM loss of 9.3 per cent.

V. (5) EXPERIMENT 02 - RESULTS.

The following fresh weights of grass were ensiled with the given treatments:-

Treatment	Silo		
A	1	3126 kg	Sealed immediately and ensiled for 139 d.
A	2	3193 kg	Sealed immediately and ensiled for 139 d.
B	3	3187 kg	Open for 72 h and sealed for 136 d.
B	4	3195 kg	Open for 72 h and sealed for 136 d.
C	5	2446 kg	Sealed immediately and unloaded after 72 h.
C	6	2424 kg	Sealed immediately and unloaded after 72 h.
D	7	2363 kg	Open for 72 h and unloaded.
D	8	2468 kg	Open for 72 h and unloaded.

The material in silo 3 collapsed and was rebuilt after 24 h. The weights of effluent at the bases of silos 1 and 6 broke the seals of these silos. After the removal of the effluent they were resealed.

V. (5) 1 Temperature changes.

The temperature of the outer layers of the 'open' silos rose steeply during the first 24 h of ensilage and readings of 61°C and 54°C were recorded just below the surface of silos 7 and 8 respectively. After 72 h the temperatures were 69°C and 68°C respectively (ambient temperature -16°C). There was little change in temperature (25°C) in the material near the bases of the 'open' silos during the first 72 h of ensilage. Maximum temperatures of 26°C and 27°C were recorded in the sealed silos 5 and 6 respectively during this period. Maximum temperatures recorded on the thermometers removed from the top and bottom of the silos after 139 d were:- silo 1, 25°C; 18°C; silo 2, 25°C; -; silo 3, 40°C, 27°C; silo, 4, 43°C, 27°C.

V. (5) 2 Composition.

The composition of the grasses ensiled in the eight silos is given in Table 9.

TABLE 9.

Composition of grasses.

Treatment	A		B		C		D	
Silo	1	2	3	4	5	6	7	8
DM (%)	14.0	15.6	15.9	16.2	15.8	16.1	18.3	16.4
pH	6.16	6.07	6.48	6.08	6.33	6.29	6.30	6.28
Bc (mequiv/100g DM)	32	32	27	37	31	28	37	29
Components of DM (%)								
OM	89.8	90.5	88.8	90.4	90.7	90.6	89.2	90.6
CP	22.9	23.9	22.9	23.8	23.7	21.8	22.2	22.8
CF	25.0	22.7	23.8	22.3	19.7	20.2	20.3	22.1
MAD - F	29.1	27.2	29.9	27.6	27.5	27.4	28.2	27.8
TN	3.67	3.82	3.67	3.80	3.79	3.48	3.54	3.65
Nitrate-N	0.41	0.39	0.41	0.42	0.40	0.31	0.36	0.31
WSC	8.7	11.0	9.7	11.7	11.5	12.4	9.1	12.2
Glucose	1.9	-	-	-	-	-	-	-
Fructose	2.5	-	-	-	-	-	-	-
Fructans	1.2	-	-	-	-	-	-	-
Oligosaccharides (including sucrose)	2.6	-	-	-	-	-	-	-
Cellulose	27.7	25.6	26.6	25.2	26.0	26.5	25.2	26.0

The compositions of the grasses ensiled in the eight silos were similar but there was an increase in DM and WSC contents throughout the day as the silos were filled consecutively. The CP contents were higher and the WSC contents lower than in experiment 01. Percentages of nitrate-N were high in all the grasses.

Dense brown fumes of nitrogen dioxide escaped from the 'sealed' silos when

they were opened after 72 h. The outer layers of the herbage from these silos resembled grass in colour but, immediately below the surface and throughout the silo, the herbage had the appearance and odour of good silage. The 'open' silos were green on the surface but below this there was a layer of dark green, slimy material which smelt strongly of ammonia. Next, there was a layer of very hot, dark brown herbage surrounding the inner core which was at a much lower temperature and which resembled the material from the 'sealed' silos in colour and odour.

The analysis of the nine samples of silage taken to study the course of fermentation at different positions in the silo after 72 h and 139 d are given in Appendix 2 (Tables A7 - A11). A statistical analysis of these results was carried out as in experiment O1 and the results of this are given in Tables 10a - 10d.

TABLE 10a.

Percentage DM.

Treatment	Total samples (18)	Position		SE of position differences
		Outside (10)	Inside (8)	
A	16.9	16.3	17.6	
B	17.4	17.6	17.2	±0.22
C	15.9	15.3	16.6	
D	15.6	14.1	17.4	
SE of treatment differences	±0.30	±0.41	±0.46	
Marginal mean	16.4 ± 0.106	15.8	17.2	

SE of differences between marginal means — ± 0.44

The DM contents of the silages after 139d were significantly higher than those of the silages after 72h but there was no significant difference between the DM contents of the inside samples after 72h and 139d. After 72h, the DM percentages of the outside samples from the 'open' silos were significantly lower than those of the outside samples from the 'sealed' silos but there was no significant difference between the values for the inside samples. In treatments A, C and D, the DM contents of the inside samples were significantly higher than those of the outside samples.

TABLE 10b.

pH

Treatment	Total samples (18)	Position Outside (10)	Position Inside (8)	SE of position differences
A	4.44	4.45	4.42	
B	5.42	5.74	5.01	±0.119
C	4.42	4.57	4.22	
D	6.28	7.86	4.31	
SE of treatment differences	±0.185	±0.238	±0.263	
Marginal means	5.14 ± 0.065	5.66	4.49	
		SE of differences between marginal means ±0.238		

The pH values of the samples from the 'sealed' silos after 72h were not significantly different from those from the 'sealed' silos after 139d and they were both significantly lower than the values for the 'open' silages after 72h and 139d. The samples from the 'open' silos after 72h had a significantly higher pH value than those from the 'open' silos after 139d, but the pH values of the inside samples of the 'open' silos after 72h were not significantly different from those of the inside samples of treatments A and C. The pH values of the inside samples in treatments B, C and D were significantly lower than those of the outside samples.

TABLE 10c.

Percentage WSC in DM.

Treatment	Total samples (18)	Position		SE of position differences
		Outside (10)	Inside (8)	
A	0.5	0.5	0.6	
B	0.7	0.6	0.8	±0.16
C	3.1	4.2	1.7	
D	1.5	1.2	1.9	
SE of treatment differences	±0.28	±0.35	±0.38	
Marginal means	1.4 ± 0.099	1.6	1.2	

SE of differences between marginal means ± 0.33

The WSC contents of the final silages were significantly lower than those of the 72 h silages. After 72 h, the 'open' silages had significantly less WSC than the 'sealed' silages. The WSC content of the outside samples followed the same pattern but there was no significant difference between the values for the inside samples at either 72 h or 139 d.

TABLE 10d.

Percentage MAD-F in DM.

Treatment	Total samples (18)	Position		SE of position differences
		Outside (10)	Inside (8)	
A	33.3	33.1	33.5	
B	36.2	39.1	32.6	±0.42
C	29.6	29.0	30.4	

TABLE 10d. (contd.)

Treatment	Total samples (18)	Position		SE of position differences
		Outside (10)	Inside (8)	
D	33.2	36.5	29.0	
SE of treatment differences	±1.08	±1.21	±1.27	
Marginal means	33.1 ± 0.382	34.4	31.4	
		SE of differences between marginal means ± 0.84		

The MAD - F contents of the 72 h 'sealed' silages were significantly lower than those of the silages from the other treatments. The MAD - F contents of the outside samples followed the same pattern but the values for the samples from treatment A were significantly lower than those from treatments B and D. There was no significant difference between the MAD - F contents of the inside samples either after 72 h or 139 d. With one exception (treatment C) the MAD - F contents of the outside samples were significantly greater than those of the inside samples.

The lactic acid contents of the bottom samples from silos 5, 6, 7 and 8 were high after 72 h but there was very little acid in the surface layers of the 'open' silos. After 139 d, acetic acid was the major acid in the surface layers of silos 1 and 2 but there was also some lactic acid in the surface layer of silo 2. Lactic acid percentages were high in the bottom layers of both silos. Acetic acid contents were high in the silages from silos 3 and 4 but lactic acid was present in the bottom layers. Butyric acid contents were higher in the 'open' silages than in the 'sealed' silages.

Quantitative measurements of the individual sugars were not made on the water extracts of the nine samples taken from each silo after 72 h but assessments of

the relative concentrations of glucose, galactose, fructose and mannitol were obtained visually from chromatographic strips. The results are shown in Table 11. Galactose and mannitol were present in most samples from silos 5 and 6 after 72h. Glucose and fructose were only present in small quantities in the material near the bottom of these silos. Similar results were obtained for the inner samples from the 'open' silos but on the surface the sugar content was negligible. Traces of xylose and arabinose were present on some chromatograms.

TABLE 11.

Individual sugars.

Treatment	Silo	Sample		Glucose	Fructose	Galactose	Mannitol
C	5	Top	1	xx	xx	tr	xx
			2	xx	xx	nil	xx
			3	-	-	-	-
		Middle	1	tr	tr	tr	xx
			2	nil	tr	tr	xx
			3	nil	tr	tr	xx
		Bottom	1	nil	x	tr	xx
			2	nil	tr	tr	xx
			3	nil	tr	tr	xx
C	6	Top	1	xx	xx	x	x
			2	xx	xx	x	xx
			3	xx	xx	x	xx
		Middle	1	xx	x	x	x

TABLE 11. (contd.)

Treatment	Silo	Sample		Glucose	Fructose	Galactose	Mannitol
C	6	Middle	2	x	x	x	xx
			3	tr	tr	x	x
		Bottom	1	xx	xx	x	xx
			2	tr	tr	x	xx
			3	tr	tr	x	xx
D	7	Top	1	nil	nil	tr	x
			2	nil	nil	nil	nil
			3	nil	nil	nil	nil
		Middle	1	xx	xx	x	x
			2	tr	x	x	x
			3	tr	tr	x	xx
		Bottom	1	nil	nil	nil	tr
			2	tr	nil	x	x
			3	tr	tr	x	xx
D	8	Top	1	nil	nil	nil	nil
			2	nil	nil	nil	nil
			3	nil	nil	nil	nil
		Middle	1	tr	tr	nil	xx
			2	nil	nil	tr	x
			3	tr	xx	xx	xx
		Bottom	1	tr	nil	nil	nil
			2	nil	nil	tr	x
			3	tr	tr	tr	nil

The composition of the bulked samples taken after 72h from silos 5, 6, 7 and 8 is given in Table 12. There was little difference between the materials of the central cores of the silos in treatment C and D and this similarity is borne out by the compositions of the bulked samples. The higher contents of MAD-F suggest that the losses of DM were greater from the 'open' silages. Nitrate-N levels were high in all the silages. A high proportion of the fructose present in the grass appeared as mannitol in the 72h silages.

TABLE 12.

Composition of the 72h silages.

Treatment	C		D	
Silo	5	6	7	8
DM (%)	16.6	16.6	16.6	16.8
pH	4.31	4.30	4.47	4.63
Bc (mequiv/100g DM)	65	59	60	62
Components of DM (%)				
MAD-F	29.5	29.9	31.5	32.1
N	3.76	3.56	3.96	3.59
Nitrate-N	0.36	0.26	0.28	0.23
WSC	1.7	3.0	2.6	1.7
Glucose	0.4	0.5	0.2	0.3
Fructose	0.7	1.3	0.9	0.9
Xylose	tr	tr	0.1	0.1
Galactose	0.2	0.4	0.3	0.1
Arabinose	tr	tr	tr	tr
Oligosaccharides (including sucrose)	0.4	0.5	0.9	0.6

TABLE 12. (contd.)

Treatment	C		D	
Silo	5	6	7	8
Fructans	0.1	0.1	0.1	0.1
Mannitol	2.6	3.4	2.2	2.3
Cellulose	27.2	26.5	27.2	27.6
Ethanol	0.32	0.37	0.31	0.29

The composition of the bulked samples taken from silos 1, 2, 3 and 4 after 139d is given in Table 13. The outer layers of silos 3 and 4 were slimy and contained patches of mouldy material. These layers were weighed and sampled separately. The analysis of the effluents is given in Table 14 and the pH values of the effluents are shown in Figure 2.

TABLE 13.

Composition of 139d silages.

Treatment	A		B			
Silo	1	2	3		4	
			Waste	Edible	Waste	Edible
DM (%)	17.6	18.7	16.2	17.4	15.2	17.8
pH	4.81	3.97	5.72	5.43	5.69	4.91
Bc (mequiv/100g DM)	109	118	-	130	-	140
Components of DM (%)						
OM	89.7	90.1	84.8	85.0	84.1	87.9
CP	23.3	23.1	-	24.1	-	24.8
CF	28.2	26.7	-	28.4	-	26.5

TABLE 13. (contd.)

Treatment	A		B			
Silo	1	2	3		4	
			Waste	Edible	Waste	Edible
MAD-F	34.1	32.6	38.9	37.1	39.4	32.1
TN	3.73	3.69	-	3.85	-	3.96
PN	1.64	1.63	-	1.67	-	1.82
NPN	2.09	2.06	-	2.18	-	2.14
VN	0.48	0.40	-	0.79	-	0.73
VN as % TN	12.8	10.9	-	20.5	-	18.4
Nitrate-N	0.06	0.19	-	0.04	-	0.05
WSC	0.3	0.5	-	0.4	-	0.7
Mannitol	0.2	0.1	-	0.3	-	0.3
Cellulose	30.7	30.0	32.7	31.3	30.1	29.6
Acetic acid	9.7	4.0	-	11.6	-	4.3
Propionic acid	0.7	0.2	-	1.4	-	0.7
Butyric acid	0.2	0.2	-	2.3	-	1.0
Lactic acid	3.4	10.5	-	tr	-	1.0
Succinic acid	tr	tr	-	0.2	-	0.1
Ethanol	0.83	0.46	-	0.88	-	0.91

Only the material from silo 2 could be said to be a well-preserved silage, of low pH value and high lactic acid content. In silo 1, the pH value of the silage was higher (4.81), and the lactic acid had been partly replaced by acetic acid. Although lactic acid was detected in the samples from the bottom layers of silos 3 and 4, the bulked silages were of poor quality, especially the material from silo 3. In each treatment, the silage of poorer quality had the lower buffering

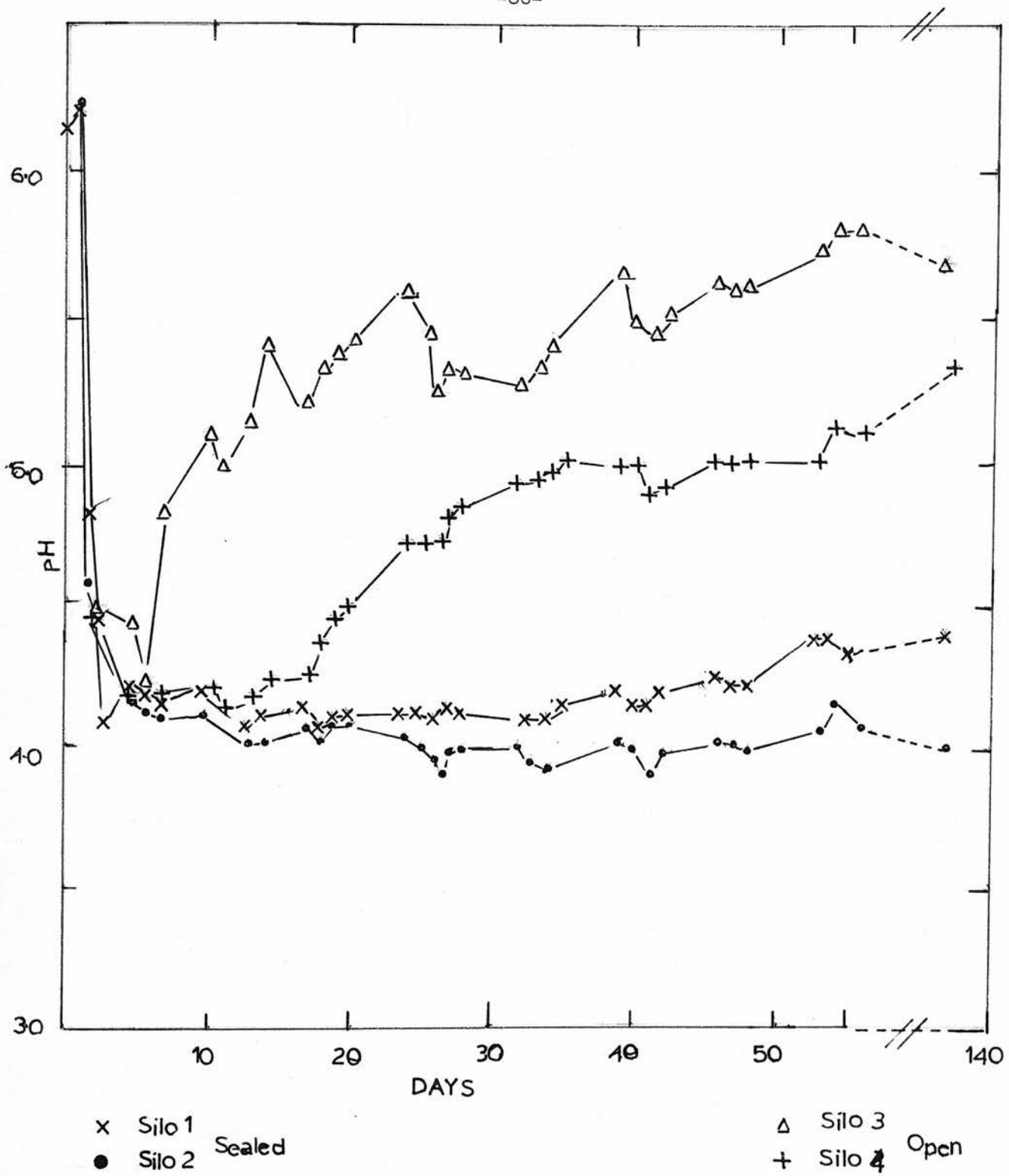


Fig. 2. Changes in effluent pH with time EXPERIMENT 02.

capacity. Butyric acid and volatile-N contents were higher in the silages from the 'open' silos. The highest MAD-F values were obtained for the waste material.

TABLE 14.

Analysis of effluents.

Treatment	A		B	
Silo	1	2	3	4
DM (%)	5.3	5.8	4.9	5.8
Components of DM (%)				
OM	75.8	76.3	80.3	74.3
N	5.8	5.1	6.2	5.8
Nitrate-N	4.3	4.1	1.5	2.8
WSC	7.9	9.8	3.5	5.2
Mannitol	13.9	13.6	4.2	11.7
Lactic acid	44.8	44.1	16.4	35.7
Ethanol	1.2	1.1	1.4	1.6

A high proportion of the products of fermentation (lactic acid and mannitol) was lost in the effluent in this experiment. The pH values of the effluents from all the silages fell rapidly to below 4.5 during the first few days. The pH values of the effluents from the 'open' silos rose gradually throughout the ensiling period but the value for the effluent from silo 4 was always lower than that of the effluent from silo 3. There was little change in the pH value of the effluent from silo 2 after the initial fall but the value for the effluent from silo 1 rose gradually after the 40th day.

V. (5) 3 Microbiological assay.

Microbial counts for six samples from the grass, as ensiled, are given in

Table 15. The counts of lactic acid bacteria on Tween acetate agar were much higher than in experiment 01. Counts of yeasts and moulds were very low. No assay was made of coliforms, lactate fermenters or proteolytic clostridia on these grasses.

TABLE 15.

Microbiological assay of grasses.

Counts/10g fresh grass.

Sample	TAA	MEA	YEA
1	2.4×10^7	4	1.5×10^7
2	2.6×10^7	7	2.6×10^8
3	6.6×10^8	20	5.5×10^8
4	3.5×10^7	10	4.7×10^8
5	3.4×10^8	10	3.7×10^8
6	2.1×10^8	20	5.9×10^8

TAA - Tween acetate agar (lactic acid bacteria)

MEA - Malt extract agar (yeasts and moulds)

YEA - Yeast extract agar (all organisms)

Counts of organisms on the nine samples of silage taken from each silo are given in Appendix 2 (Table A12). Total counts and counts of lactic acid bacteria were similar in the four silos opened after 72h. In general, microbial proteolytic and lactate fermenting activities were higher in the 'open' silages but counts of proteolytic clostridia were high in the surface material from silo 5. Yeast and/or mould activity was highest in the silage at the bottom of the 'sealed' silos. At the end of the ensiling period, moulds were detected in the material on the top surface of silo 1 and in the material at the centre of silos 3 and 4. Counts of lactic acid bacteria were lowest in the silage at the bottom

of silo 2. The only indication that the silage from silo 1 was of poorer quality than the silage from silo 2 was the higher count of proteolytic clostridia in silage 1. Counts of proteolytic clostridia were also high in the silages from silos 3 and 4.

V. (5) 4 Losses.

The weights (kg) of fresh silage and effluent removed from the silos were:-

Treatment	Silo	Silage		Effluent
		Waste	Edible	
A	1	nil	2061	552
	2	nil	2335	754
B	3	534	1599	373
	4	595	1782	237
C	5	nil	2084	-
	6	nil	2147	-
D	7	-	1973	-
	8	-	1993	-

Waste and edible materials were not separated from silos 7 and 8 and effluent loss was not measured from silos 5, 6, 7 and 8. Losses of DM from the silos are given in Table 16 and percentage losses of some components of DM are given in Table 17.

TABLE 16.

Percentage DM losses during ensilage.

Treatment	A		B		C		D	
Silo	1	2	3	4	5	6	7	8
Fermentation and oxidation	10.7	3.6	17.7	16.0	-	-	-	-
Effluent	6.7	8.8	3.6	2.6	-	-	-	-
Total DM loss	17.4	12.4	21.3	18.6	10.6	8.8	24.1	17.3
Total edible loss	17.4	12.4	44.9	39.0	-	-	-	-
DM losses calculated from terylene bags	9.8	6.9	20.0	36.4	8.0	3.0	21.0	13.4
Total edible loss calculated from terylene bags.	9.8	6.9	43.1	50.2	-	-	-	-

The fermentation and oxidation losses from the 'sealed' silos, 1 and 2, are lower than those of the silages treated in a similar manner in experiment 01. The value for the silage from silo 2 (3.6 per cent) lies within the calculated values obtained from the biochemical pathways for the fermentation of WSC to organic acids. The higher DM losses from silos 1 and 3, relative to those from silos 2 and 4, reflect the poorer quality of these silages. Calculated from grass input and silage output the total edible loss was greater from silo 3 than that from silo 4.

TABLE 17.

Percentage losses of DM components from the 139d silages.

Treatment	A				B			
Silo	1		2		3		4	
	Silage	Effluent	Silage	Effluent	Silage	Effluent	Silage	Effluent
OM	17.4	5.6	12.8	7.4	24.6	4.1	20.9	2.2
WSC	97.1	6.0	96.0	7.8	96.8	1.3	95.1	1.2
MAD-F	3.1	nil	+5.0	nil	2.3	nil	5.7	nil
Cellulose	8.4	nil	+2.6	nil	7.3	nil	4.4	nil

V. (6) DISCUSSION.

Although the grass ensiled in this experiment was a third cut from the same field as that used in the previous experiment, the fermentation patterns of the silages in experiments 01 and 02 were quite different. The application of a nitrogenous fertiliser to the grass, two weeks before cutting, suppressed the DM and WSC contents and increased the levels of CP and nitrate. Jones (1961) noted that the highest levels of nitrate content of grasses were attained in the autumn cuts and that there was a close inverse relationship between nitrate content and WSC content. Sprague and Taylor (1970) applied 28 kg, 140 kg and 390 kg N/ha to Dactylis glomerata and found that the WSC percentage was halved and the CP percentage almost doubled from the low to the high rate of application of fertiliser. From their ensiling experiments, they concluded that the WSC/CP balance in plant tissue at the time of harvest regulates bacterial growth and so determines the observed chemical changes and losses during fermentation. Volatile acid levels were always high with the high levels of N application and pH values were low at the low levels of N application. Wilson (1969) noted

that the application of high levels of nitrogenous fertiliser to pasture grass suppressed the DM content of the silage but increased the lactic acid content, indicating a more vigorous activity of the lactic acid bacteria on the herbage of high CP content.

During the early stages of experiment 02, there was a more rapid fermentation of WSC than during the same period in experiment 01. After 48h, the pH values of the effluents from the 'sealed' and 'open' silages had fallen well below 5 and a concentration of 10 per cent of lactic acid in the DM was found in one sample of silage after 72h. The maximum temperatures in the 'sealed' silos ($27^{\circ}C$) at that time were several degrees higher than those recorded in the corresponding silos in experiment 01 and the herbage had the colour and odour of good silage. When Sprague and Taylor (1965) ensiled Dactylis glomerata grown with low and high levels of nitrogen fertility, the recorded temperatures near the centre of the plastic silos were several degrees higher in the grass grown with the high level of nitrogen fertility and the rate of fall of pH value was more rapid in these silos. Temperatures at the surface of the 'open' silos were a few degrees lower than in experiment 01 but this would be expected as the DM was lower with consequently higher specific heat giving a lower temperature rise for the same amount of heat produced. After 72h, there was no statistical difference in DM, pH, WSC or MAD-F percentages between the inside samples from the two treatments (C and D) and lactic acid contents were high in the samples analysed. Approximately 80 per cent of the WSC in the grass as ensiled 'disappeared' from the herbage during the first 72h of ensilage. This sugar possibly augmented with the breakdown products of higher carbohydrates, resulted in the high lactic acid and mannitol contents of the 72h silages. The differences between treatments which had occured during the first 72h appeared in the material of the surface layers. With the exception of the relatively

high WSC content of the outer layers of the 'sealed' silos there was little difference in composition within the silos. Immediately below the green surface layer of the 'open' silos was a layer of slimy material smelling strongly of ammonia and below this a 'hot' area of dark brown material. The outside samples of silage taken from these silos were of high pH value and high MAD-F content.

At the end of the ensiling period, the WSC and mannitol levels were low in all the silages. Some of the mannitol present in the herbage after 72h was recovered in the effluent but the remainder may have been used as substrate by the lactic acid bacteria (Whittenbury, 1961). The silage from one of the 'sealed' silos (silo 2) had a low pH value (3.97) and a high lactic acid content (10.5 per cent of DM). The silage from silo 1 was poorer in quality and this may have been caused by one or more of several factors. The grass in this silo was the first cut of the day and the DM and WSC contents were at their lowest, drainage from the silo was poorer than that from silo 2 and, as there was no visible build - up of gas in this silo during the first few days of ensilage, it may have been leaking, possibly at the seam. Whatever the cause, the silage had a higher pH value (4.81), higher acetic acid content (9.7 per cent DM) and lower lactic acid content (3.4 per cent DM) than the silage from silo 2. The high lactic acid content of the effluent from silo 1 and the lactic acid content of the sample of silage taken from the bottom layer of the silo (13.0 per cent DM) suggest that there was no difference in the fermentation pattern in the two silos initially. Butyric acid concentrations were low in both silages. Langston et al (1962) found that aeration of a silo increased the acetic acid content of the silage, and when Gordon et al (1969) ensiled first cut Medicago sativa in 0.025 mm thick polythene bags and in glass jars, the material in the polythene bags underwent an acetic acid fermentation but the material in the glass jars underwent a normal lactic acid fermentation. The difference was attributed to the porosity of the

polythene. It is interesting to note that the sample from the top surface of silo 2 also had a high acetic acid content (7.5 per cent DM). Fermentation and oxidation losses were much higher in silo 1 and pH values and MAD-F percentages of the material from the outer layers of this silo indicate that the higher losses occurred in this area.

There were within-treatment differences in quality and composition also between the silages from silos 3 and 4, possibly due to excessive oxidation in silo 3 when it collapsed and was rebuilt after 24h. Acetic acid values were again high in the poor silage but in these silages there had also been a clostridial fermentation. This would be encouraged by the high pH values at the surface of silos 3 and 4 when they were sealed. Butyric acid levels were higher and lactic acid levels lower in the material from silo 3. Nilsson et al (1956) claimed that, in silage fermentation of high CP low WSC crops, the temperature greatly influences the quality of the silage. When an ensiled grass/clover mixture was held at 5°C and 37°C, the pH values after one week were 4.9 and 4.2, respectively, but after 24 weeks they were 4.7 and 5.4. At the higher temperatures the WSC and CP broke down more rapidly. The ammonia produced neutralised the lactic acid, and the low WSC content was insufficient to produce adequate quantities of lactic acid to keep the pH value down. At 2°C the volatile N content was only 4.9 per cent of TN, but at 37°C it was 63.4 per cent of TN. Wieringa et al (1961) stated that, under strictly anaerobic conditions, the optimum temperature for butyric acid fermentation is 35°C and for putrefaction it is 30°C and that both optima are higher when the material is aerated. Grass of low WSC content (4.3 per cent DM) produced a good quality silage at all temperatures but when aerated for one or two days, lactic acid production decreased and there was an increase in the concentration of ammonia, butyric acid and acetic acid. In the 139d silages of experiment 02, butyric acid and ammonia contents were low in the 'sealed'

silages which reached a maximum temperature of 27°C. In the 'open' silages, with a temperature gradient ranging from 69°C in the surface layer to 25°C near the base, ammonia and butyric acid production was greater than in the 'sealed' silos, the high butyric acid contents occurring in the 'high temperature' material.

When Weise (1971) ensiled grass in small laboratory silos (capacity 120 ml), sealing one set immediately and the other set after 72h, there was little difference in the rate of fall of the pH value between the two types of silage but the 'sealed' silages fell to a lower pH value (4.5) than the 'open' silages (4.8) and lactic acid contents (11.0 per cent of DM) of the 'sealed' silages were higher than those of the 'open' silages (6.5 per cent DM). Due to within-treatment differences, the DM, WSC and MAD-F contents of the inside samples of treatment B were not statistically different from those of treatment A, although the pH values were higher and the lactic acid contents lower in the 'open' silages. The differences in composition were again more evident in the outer layers, with the DM, pH and MAD-F contents of the samples from treatment B statistically higher than those from treatment A. Only the WSC contents, which were very low, showed no significant difference between treatments.

The similarity in counts of lactic acid bacteria in the silos opened after 139d confirms the theory of Kempton and San Clemente (1959) that the counts of lactic acid bacteria in a silage give no indication of the quality of the silage. Counts on the ensiled grass were much higher than in experiment 01, but at 72h, there was little difference in the assays for the two experiments. There was a considerable difference between the counts on malt extract agar for the two grasses but it is not known whether this affected the fermentation pattern, although ethanol contents, perhaps from yeast activity, were higher in experiment 01. Production of gases during the first few days of ensilage were similar in both experiments. Although volumes could not be measured it is possible that these

approached the values quoted by Honig (1968) i.e. 2,500 l/100kg DM at 15 per cent DM to 700 l/100kg DM at 50 per cent DM. Acetic acid contents were higher after 72h than in experiment 01, but as the lactic acid fermentation had progressed further in experiment 02, it is impossible to say to what extent this was the result of the activity of the coliform bacteria. Weise (1971) studied the bacteria present on silages sealed immediately and aerated for 72h during a period of 90d of ensilage and obtained marked differences in bacteriological counts. With aeration lactic acid bacteria did not multiply to the same extent, counts of coliform bacteria remained high until the 16th day, proteolytic clostridia increased initially and counts of saccharolytic clostridia were still increasing after 90 days. Counts of yeasts were consistently higher in the 'open' silages throughout the ensiling period. In experiment 02, there was little difference in counts of lactic acid bacteria between treatments after 72h, with the possible exception of the higher numbers on the material from silo 6. Counts of yeasts and moulds were slightly higher in the 'sealed' silos than in the 'open' silos. At the end of the ensiling period, counts of proteolytic clostridia were higher in the 'open' silages than in the 'sealed' silages.

Barnett (1953) reported a temporary inhibition by potassium nitrate on the formation of lactic acid in slurries made from minced grass and water. He claimed that inhibition was due to the formation of nitrite which was then decomposed to nitric oxide. Wieringa (1966) stated that ensiling grass at a young stage of growth, when high rates on nitrogenous fertiliser have been applied, may lead to an abnormal fermentation as nitrite inhibits butyric acid production. In Wieringa's experiments, medium concentrations of nitrate (0.11 - 0.20 per cent of fresh grass) proved advantageous. Workers at Beltsville (1969) and Honig (1968) stated that the bacterial reduction of nitrate to nitric oxide or nitrogen dioxide occurs during the first few days of ensilage. In experiment 02, nitrate levels were high in the grasses (approximately 0.3 per cent of the fresh grass) but there was no apparent

inhibition of lactic acid bacteria and the level of nitrate was higher than that at which Wieringa found inhibition of butyric acid production. Although dense brown fumes of nitrogen dioxide were released from the 'sealed' silos after 72h, there was a relatively high concentration of nitrate-N in the silages at this time and in the material from silo 2, the only well-preserved silage at the end of the ensiling period. Stanier et al (1963) claimed that the ability to reduce nitrate to nitrite is possessed by many bacteria in the absence of oxygen but the ability to reduce nitrate beyond the nitrite stage is possessed by only a few. Barnett (1953) claimed that the organisms which reduce nitrate to nitrite are facultative in character and the reduction occurs to a lesser extent under anaerobic conditions.

Jones (1970a) stated that there is no advantage in ensiling grass of high digestibility and high CP content if the fermentation is poor and the silage is unacceptable to stock. The results of experiment 02 illustrate forcibly the dangers of ensiling a crop on a commercial scale too soon after a heavy application of nitrogenous fertiliser. Ensiled on an experimental scale in an air-tight container, this grass had the potential to produce a good silage in terms of low pH value and high lactic acid content. It took only a small variation in the chemical composition of the grass or, more probably, a slight air-leak in the container to change the character of the silage. Delayed sealing, not uncommon on the farm, had a deleterious effect, changing the chemical composition of the silage and decreasing the production of edible material by one third on the experimental scale.

VI. FORMIC ACID AS A SILAGE ADDITIVE.

VI a. THE EFFECT OF FORMIC ACID ON THE FERMENTATION OF GRASS OF LOW DM AND LOW WSC CONTENTS.

VI a. (I) INTRODUCTION.

It has been established by many workers that a minimum level of WSC in herbage is required to guarantee a satisfactory fermentation during ensilage. Although a hexose content of 6 - 7 per cent of the DM has been suggested as sufficient to lower the pH value to 4.0 in the silage (Smith, 1962), a figure of 10 per cent WSC in the DM is probably more realistic and the absolute value will vary with the crop and the conditions under which it is ensiled. When crops may prove difficult to ensile, the use of an additive is recommended and, in recent years, there has been renewed interest in formic acid, first used by Dirks in 1926. The efficiency of the additive was increased with the introduction of the flail -type forage harvester and the applicator designed by Aas et al (1966).

Although formic acid has been used in Norway and other European countries for a number of years, it was not available in the U.K. on a commercial scale until 1968 when the proprietary product 'ADD-F'* (85 per cent formic acid) came on the market. The recommended rate of addition is 0.5 gal/ton (2.21 ml/kg) or 0.265 per cent of the fresh matter applied directly to the herbage from a container attached to the forage harvester.

The grasses ensiled in the five experiments discussed in this section were low in DM and WSC contents. In experiments F1, F2 and F3, three levels of formic acid were examined (0.23 per cent, 0.34 per cent and 0.51 per cent of the fresh matter) and detailed chemical changes and losses were recorded. Experiments F4 and F5 were designed to study the effect on fermentation of applying formic acid to the upper surface layer of grass in the silo.

* Manufactured by BP Chemicals International Ltd.

VI a. (2) EXPERIMENTAL

The silos used in experiments FI, F2 and F3 were the four metal tower silos described in the Techniques section. In the first and second experiments, conditions of filling were similar to those reported but in experiment F3, an attempt was made to simulate conditions on the surface of a farm silo and the grass was not consolidated with stone blocks, but only covered with plastic sheeting. The formic acid was applied to the grass in the form of 'ADD-F' from a polyethylene container attached to the forage harvester. In addition to the large silos, laboratory tower silos (capacity 1,200g) and test-tube silos (capacity, 80g) were filled with similar herbage in experiments FI and F2 and test-tube silos in experiment F3. The laboratory tower silos were attached to a series of test-tubes containing a saturated solution of barium hydroxide which absorbed the CO_2 produced during ensilage. The test-tube silos were fitted with mercury seals and opened at intervals throughout the ensiling period. In experiment FI, separate test-tube silos were set up for microbiological assay. In experiments F4 and F5, test-tube silos only were filled. In these experiments, formic acid was applied to the grass in a thin stream from a microburette and the grass was well mixed before it was ensiled in the test-tubes.

In experiment FI, the grass was pretreated with a compound fertiliser (23 per cent N, 5per cent P, 9 per cent K) applied at the rate of 314 kg/ha on both 14 April and 29 April 1968. On 4 June 1968, the grass of composition timothy (Phleum pratense) 69 per cent: meadow fescue (Festuca pratensis) 26 per cent: perennial ryegrass (Lolium perenne) 5 per cent, was cut with a flail - type forage harvester and ensiled immediately. The silos were opened 63 days after being filled.

In experiment F2, the autumn cut of grass was taken from the same field as that used in the first experiment and had previously received a dressing of commercial ammonium nitrate (34.5 per cent N) on 16 August 1968, at the rate of 292 kg/ha. On 23 September 1968, the grass (Festuca pratensis) 66 per cent:

:Lolium perenne 25 per cent :Phleum pratense 6 per cent : others 3 per cent was cut with a flail - type forage harvester and ensiled immediately. The silos were opened 125 days after being filled.

In experiment F3, cocksfoot grass (Dactylis glomerata) which had received 544 kg/ha of a compound fertiliser (23 per cent N, 5 per cent P, 9 per cent K) applied on 9 April 1969, was cut with a flail - type forage harvester on 27 May 1969 and ensiled immediately. The silos were opened 163 days after being filled. Temperature measurements were recorded by thermocouples buried at different levels throughout the herbage. Ambient temperatures were recorded by two thermocouples suspended above each silo. Methods of sampling the grasses and silages are described under Techniques and details of analytical methods used are given in Appendix 1.

Production of effluent was deliberately restricted in all three experiments in order to avoid excessive loss of formic acid, particularly in the early stages of ensilage. In the first and second experiments, free drainage was allowed after 14 days and seven days respectively. In the third experiment, free drainage was allowed after 14 days from the control silos but the flow of effluent from the treated silages was restricted for 93 days to give similar volumes from all silages.

In experiment F4, Dactylis glomerata was cut with shears and ensiled in test - tube silos and in experiment F5, Dactylis glomerata was cut with a mower and ensiled in test - tube silos. In both experiments, formic acid was applied uniformly to the grass and also at a high concentration to the surface layer and thesilos were stored both with and without mercury seals. In experiment F5, the surface of the grass was covered with a disc of plastic.

VI a.(3) EXPERIMENT F1 - RESULTS.

The following fresh weights of grass were ensiled with the given treatments:-

Silo A - 1198 Kg - Control

" B - 1198 kg - Control

" C - 1164 kg - Treated with 0.23 per cent formic acid

" D - 1164 kg - Treated with 0.23 per cent formic acid

In addition to the test - tube silos and laboratory tower silos filled with similar material, two laboratory tower silos were filled with similaar grass treated with 0.69 per cent formic acid.

VI a. (3) 1. Temperature changes.

There was an immediate rise in temperature in the control silos; 19°C was recorded on the third day. The temperature of the treated silages remained constant (16°C) until the eighth day. There was then a gradual rise until, on the 14th day, maximum temperatures of 20°C in the control silages and 21°C in the acid-treated silages were attained.

VI a. (3) 2. Composition.

The composition of the grasses and silages is shown in Table 18. Formic acid contents of the grasses, quoted in this and subsequent experiments, are the theoretical values calculated from the weight of formic acid applied to the grass in the field. The WSC value of the formic acid - treated grass was higher than that of the control material. Nitrate - nitrogen contents of the grasses were high and are, presumably, a reflection of the heavy dressing of nitrogenous fertiliser. All silages had a low WSC content and contained lactic acid. Silage A had the lowest pH value (4.30), corresponding to the highest lactic acid content (10.0 per cent). Silage B contained only 5.1 per cent lactic acid which is reflected in the higher pH value (4.72). Ethanol contents of the control silageswere less than half of those of the treated silages.

TABLE 18.

Composition of grasses and silages.

	Grasses		Silages			
Treatment	Control	Formic acid-treated (0.23%)	Control		Formic acid-treated (0.23%)	
Silo	AB	CD	A	B	C	D
DM (%)	13.70	14.53	15.37	15.05	15.90	16.49
pH after maceration	6.00	4.75	4.30	4.72	4.36	4.42
Bc (mequiv/100g DM)	30	34	105	114	96	88
Components of DM (%)						
OM	89.6	91.7	92.0	88.1	90.6	90.9
CP	23.1	23.4	21.3	22.5	22.7	23.0
CF	24.4	24.5	27.0	25.8	27.2	27.8
TN	3.70	3.74	3.42	3.59	3.63	3.67
Protein N	2.81	2.80	1.16	1.38	1.49	1.70
NPN	0.89	0.94	2.26	2.21	2.14	1.97
VN	0.04	0.04	0.32	0.51	0.30	0.35
VN as % TN	1.1	1.1	9.4	14.2	8.3	9.5
Nitrate - N	0.23	0.26	0.14	0.07	0.16	0.14
PN as % TN	75.9	74.9	33.9	38.4	41.0	46.3
WSC	8.3	11.9	0.7	0.6	0.8	0.6
Glucose	2.7	3.4	nil	nil	nil	nil
Fructose	3.0	4.0	nil	tr	nil	nil
Xylose	nil	nil	tr	tr	tr	tr
Galactose	nil	nil	tr	tr	tr	tr
Arabinose	nil	nil	tr	tr	tr	tr
Oligosaccharides (including sucrose)	1.2	1.9	0.2	0.1	0.2	0.1

TABLE 18. (contd.)

	Grasses		Silages			
Treatment	Control	Formic acid-treated (0.23%)	Control		Formic acid-treated (0.23%)	
Silo	AB	CD	A	B	C	D
Fructans	1.9	2.1	tr	tr	tr	tr
Mannitol	nil	nil	0.1	0.1	0.2	0.4
Cellulose	27.9	27.4	30.7	28.5	30.6	30.1
Lignin	3.3	3.3	3.7	3.6	3.7	3.7
Formic acid	-	1.5	nil	nil	1.4	1.4
Acetic acid	-	-	6.1	7.5	4.9	4.5
Propionic acid	-	-	nil	nil	0.1	0.2
Butyric acid	-	-	nil	tr	nil	nil
Lactic acid	-	-	10.0	5.1	5.6	4.1
Succinic acid	-	-	nil	nil	nil	nil
Ethanol	-	-	1.1	1.3	2.5	2.7

The composition of the effluents is given in Table 19 and the change in effluent pH value with time is shown in Figure 3. The highest lactic acid content was in the effluent from silo A and the WSC and ethanol contents of the effluents from the treated silages were higher than those of the effluents from the control silages. The pH values of the effluents from the control silages fell below those from the treated silages on the third day and remained lower throughout the ensiling period.

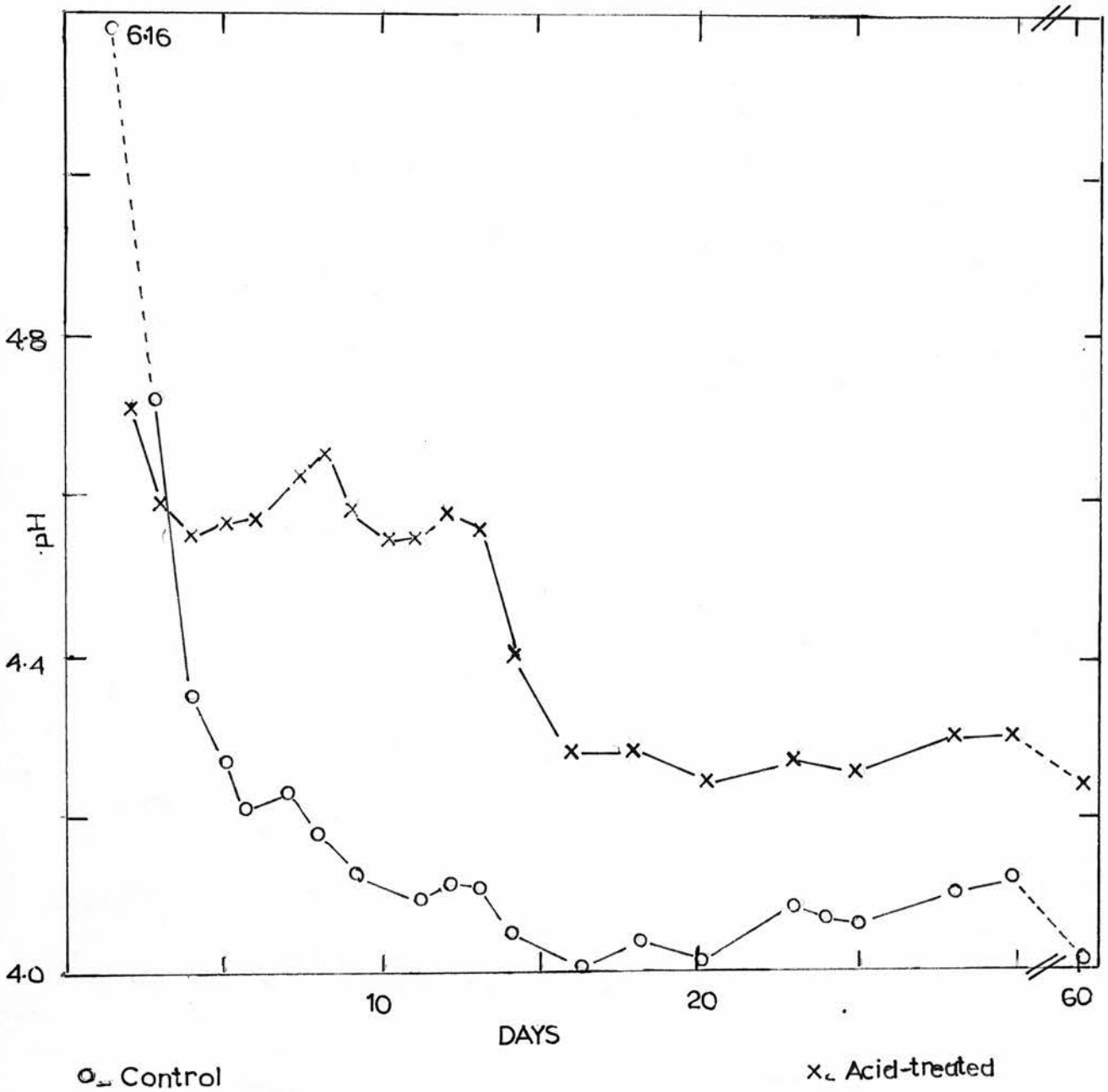


Fig. 3. Changes in effluent pH with time EXPERIMENT F1.

TABLE 19.

Composition of effluents.

	From control silages.		From formic acid - treated silages (0.23%)	
Silo	A	B	C	D
DM (%)	5.32	5.44	4.61	4.58
Components of DM (%)				
OM	82.1	82.2	78.9	79.3
N	6.2	6.0	7.0	7.1
WSC	7.2	6.7	12.6	12.2
Formic acid	nil	nil	5.8	5.7
Acetic acid	6.1	5.5	4.6	5.4
Propionic acid	0.2	0.4	0.3	tr
Butyric acid	nil	nil	nil	nil
Lactic acid	33.4	23.5	12.0	14.4
Succinic acid	0.3	0.3	nil	nil
Ethanol	1.3	1.2	10.8	11.3

VI a. (3) 3 Microbiological assay

A microbiological assay was carried out using test - tube silos filled with grass similar to that used in the main experiment. Test - tubes were opened one, two, seven and 15 days after ensiling and results of the assay are given in Table 20.

TABLE 20.

Microbiological assay of grasses and silages.
(counts/10g fresh herbage)

Treatment	Control				Formic acid - treated (0.23%)			
Sample	TAA*	YEA	LF	PC	TAA	YEA	LF	PC
Grass	3.5×10^5	5.0×10^7	$>10^3$	3.0×10^5	-	-	-	-
1-day silage	7.5×10^8	1.4×10^9	10^2	1.0×10^7	2.0×10^6	1.0×10^6	$<10^2$	2.0×10^2
2-day silage	1.9×10^{10}	8.0×10^9	$<10^2$	2.0×10^5	2.4×10^7	6.5×10^7	$<10^2$	5.0×10^5
7-day silage	-	7.5×10^8	$<10^2$	1.0×10^4	1.0×10^7	1.7×10^8	$<10^2$	1.0×10^7
15-day silage	4.2×10^8	1.0×10^8	$<10^2$	1.0×10^4	4.0×10^7	9.3×10^7	$<10^2$	1.0×10^4

* TAA - Tween acetate agar

YEA - Yeast extract agar

LF - Lactate - fermenting clostridia

PC - Proteolytic clostridia

In the control silages the micfoflora developed normally but in the treated silages, all bacterial growth was inhibited initially. After two days, proteolytic clostridia increased to similar levels to those found in the control materials. Proteolytic clostridia had decreased to low levels in both treatments after fifteen days.

VI a. (3) 4 Losses.

The weights (kg) of fresh silage and effluent removed from the silos were:-

		Silage		Effluent
Treatment	Silo	Waste	Good	
Control	A	20	902	266
Control	B	31	898	258
Formic acid - treated (0.23%)	C	23	872	256
	D	16	873	262

The increase in gaseous losses during the ensiling period, as a percentage of the DM ensiled, is shown in Figure 4. Losses from the control silages were low but increased gradually until the end of the ensiling period. There was a high gaseous loss from the treated silages between the eighth and 14th days. Otherwise the losses followed the same pattern as those from the control silages.

Detailed DM losses are given in Table 21 and losses of some individual components are given in Table 22.

TABLE 21.

Percentage DM losses during ensilage.

Treatment	Control		Formic acid - treated (0.23%)	
Silo	A	B	C	D
Fermentation and oxidation	5.0	6.5	9.0	5.6
Effluent	8.6	8.5	7.0	7.1
Waste	1.9	2.7	2.2	1.6
Total edible loss	15.5	17.7	18.2	14.3

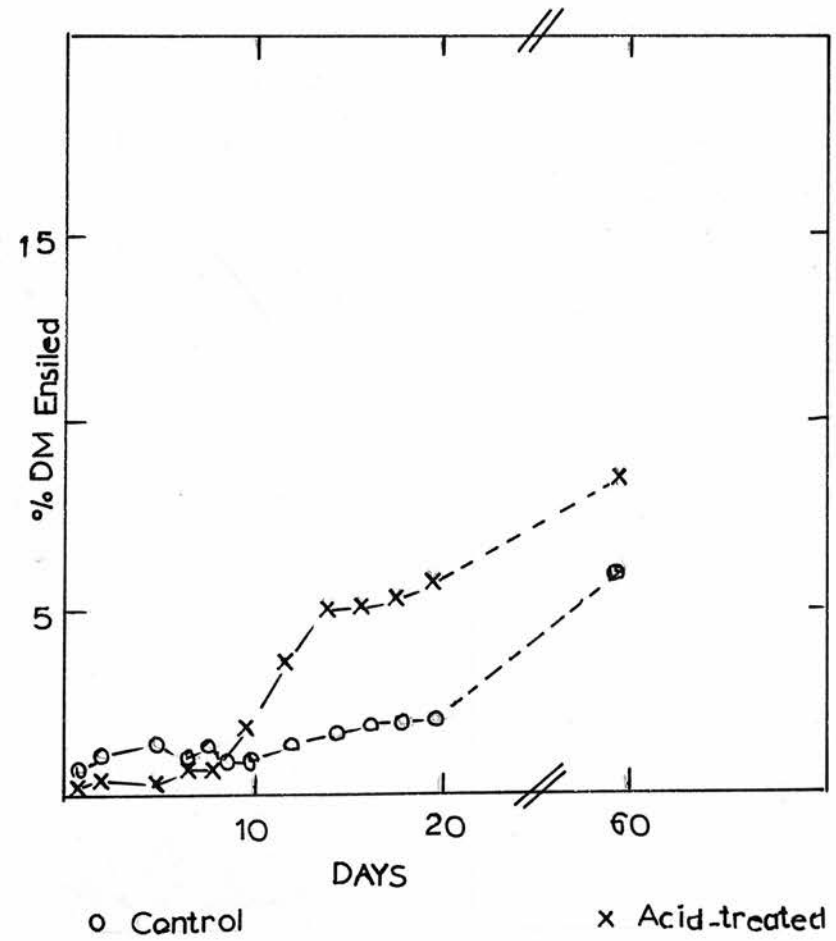


Fig. 4. Gaseous loss as percentage grass DM ensiled

EXPERIMENT F1.

TABLE 22.

Percentage losses of DM components.

Treatment	Control				Formic acid - treated (0.23%)			
Silo	A		B		C		D	
	Total	Effluent	Total	Effluent	Total	Effluent	Total	Effluent
OM	11.4	7.9	16.4	7.8	17.0	6.0	14.2	6.1
N	20.3	14.5	17.4	13.8	18.4	13.0	14.8	13.4
WSC	92.8	7.4	93.5	6.8	94.3	7.3	95.9	7.3
Formic acid	-	-	-	-	22.9	26.4	20.5	26.4
Cellulose	4.8	-	13.1	-	6.3	-	4.9	-

There were no marked differences between treatments in this experiment. The formic acid lost from silages C and D was recovered in the effluents.

VI a. (3) 5 Laboratory silos.

Changes in pH and WSC content during ensilage were examined by opening tubes at intervals throughout the ensiling period. The results are shown in Figures 5 and 6 respectively. After an initial rise, the WSC content of the control silages fell rapidly, with a similar fall in pH value. After remaining steady during the first few weeks, the pH rose to a value similar to that of control silage B. The pH values of the test - tube acid - treated silages fell gradually to pH 4.0 and then remained constant until the end of the experiment.

The results of the analysis of the silages from the laboratory tower silos (mean of two) and the losses and CO_2 production during ensilage are given in Table 23.

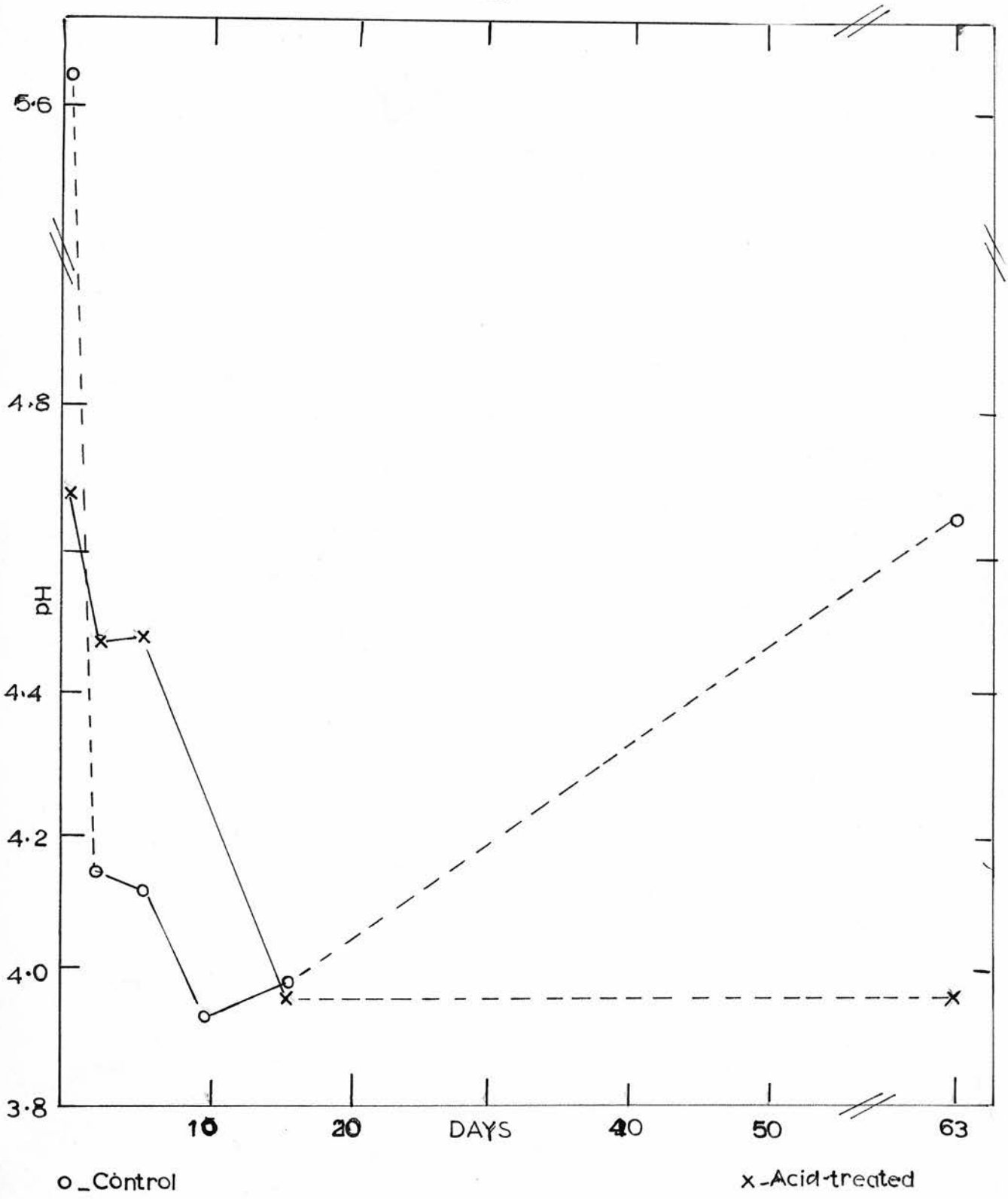


Fig. 5. pH values for laboratory tube silos EXPERIMENT F1.

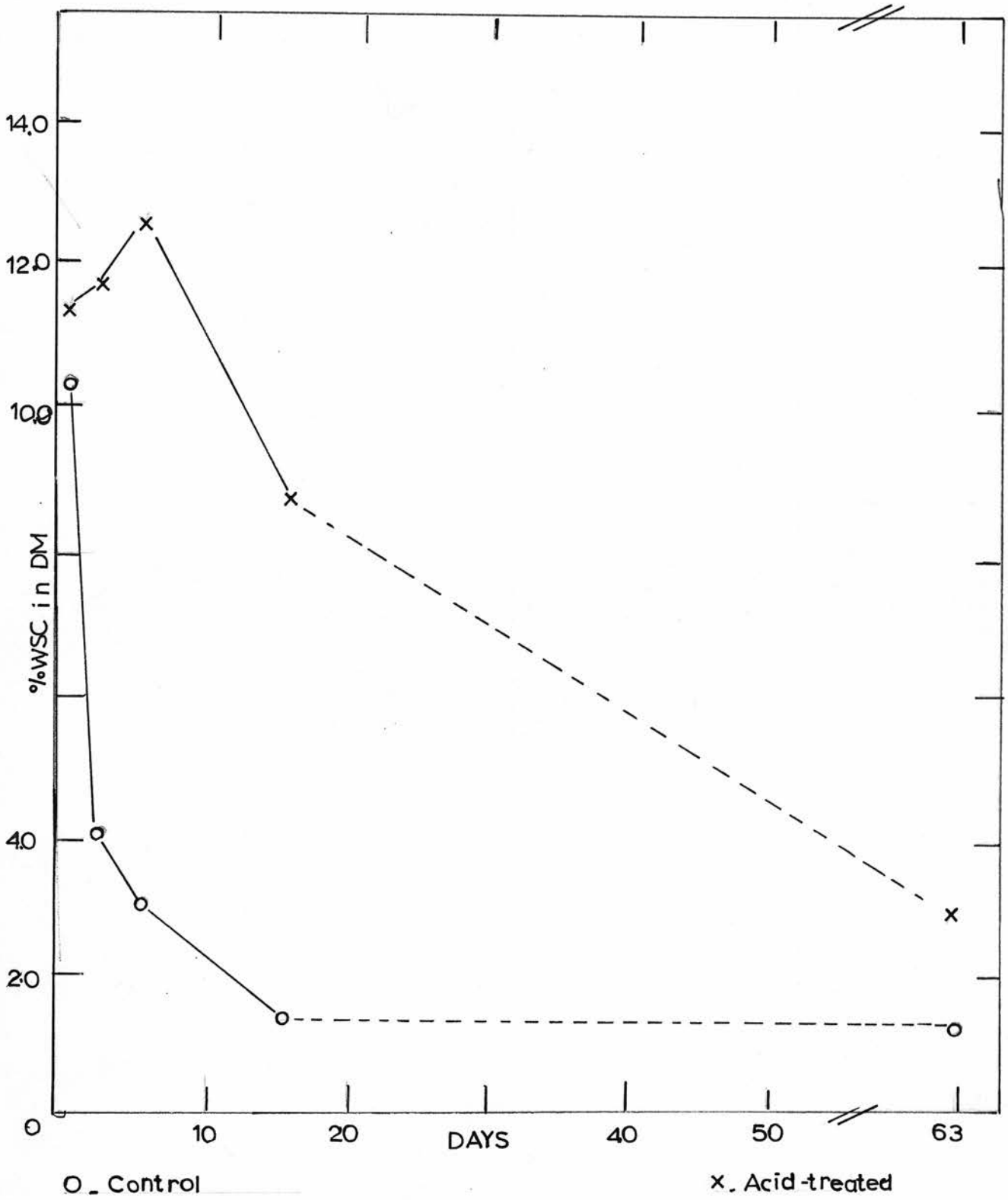


Fig. 6. WSC values for laboratory tube silos EXPERIMENT F1.

TABLE 23.

Analysis of silages and losses of DM from the laboratory tower silos.

Treatment	Control	Formic acid-treated (0.23%)	Formic acid-treated (0.69%)
DM(%)	13.1	14.0	14.2
pH	4.68	4.02	3.80
Components of DM(%)			
WSC	0.4	3.2	11.6
Formic acid	-	1.4	5.6
Acetic acid	7.7	3.0	2.0
Propionic acid	0.7	0.2	0.9
Butyric acid	tr	nil	nil
Lactic acid	7.3	10.0	2.0
Succinic acid	tr	tr	nil
Ethanol	1.2	0.4	0.3
Losses			
Fermentation and oxidation(%)	6.2	nil	nil
CO_2 absorbed (%DM ensiled)	0.3	0.2	0.05

The control silage from the test-tube silos opened at the end of the ensiling period and the control laboratory tower silages resembled silage B in pH value and composition. The treated silages, at the end of the ensiling period, were low in pH value and high in lactic acid content, but the material treated with 0.69 per cent formic acid had a low percentage of lactic acid (2.0) and a high percentage of residual WSC (11.6). Ethanol contents were low in the acid-treated silages.

VI a. (4) DISCUSSION.

In experiment 02, it was apparent that when the WSC content of herbage is low (<10 per cent of DM) small differences in composition or in efficiency of ensiling and sealing of the silos may result in silages of varying composition and quality. This was again illustrated in experiment F1. A previous experiment using the metal tower silos (McDonald et al, 1960) showed that when material of 15.1 per cent WSC content in DM was ensiled, silages similar in quality and composition were obtained. In experiment F1, however, the silage from silo A was of lower pH value and higher lactic acid content than the silage from silo B. As in experiment 02, the main differences in composition were in the acetic and lactic acid fractions. The differences in this experiment cannot be attributed to aeration as the material from the sealed test-tube and laboratory tower silos resembled the poorer material, but may have resulted from differences in compaction of the grass in silos A and B. The instability of the control silages is seen in the effluent data (Fig 3) and test-tube silages. The pH value of both fell below 4.0 but rose again after 10-15 days. The absence of butyric acid from the effluents and silages confirms that the rise in pH value was not due to the activity of saccharolytic clostridia, but the volatile-N content of silage B suggests that proteolytic clostridia were active and a longer ensiling period may have led to further degradation. Herbage similar to the type used in this experiment should benefit from the application of an additive such as formic acid.

The immediate action of formic acid was to lower the pH value of the grass to 4.75 and to reduce loss of WSC, presumably due to respiration, occurring between the field and the silo. In order to prove that the additional WSC in the treated grass did result from the inhibition of respiration, young grass

of 14.5 per cent DM was cut and a sample taken for analysis. One half of the remainder was sprayed with formic acid and the other was kept untreated as a control. After four hours the grasses were sampled and analysed for WSC content. The initial percentage of WSC in the DM was 8.0 and this fell to 7.3 per cent in the treated grass and 5.7 per cent in the control grass. In addition to inhibiting respiration, formic acid inhibited all bacterial growth during the early stages of ensilage. The pH value of the treated material in the test-tube silos fell more slowly than that of the control silages and remained at pH 4.0 until the end of the ensiling period. The pH value of the effluent from the treated material in the metal tower silos fell sharply after the tenth day but levelled off at pH 4.3 and rose gradually after the 20th day. About the tenth day there was an increase in the gaseous loss and in the temperature of these silages. The lactic acid contents were lower and the acetic acid contents higher in these silages than in the corresponding laboratory tower silages, possibly due to the removal of formic acid in the effluent from these silos. At the higher level of application of formic acid, lactic acid bacteria were further inhibited, resulting in a low level of lactic acid and high residual WSC in the silages.

The volatile-N values for silages C and D confirm the results of the microbiological assay that formic acid controlled the proteolytic clostridia. Proteolysis was also less in these silages. Nitrate-N determinations were not carried out on the effluents in this experiment but losses from the silages were less than 50 per cent in silos A, C and D. The higher loss from silo B is in agreement with the findings of other workers (Wieringa, 1966).

The treated silages from the laboratory tower silos differ from the material from the metal tower silos in their ethanol contents and in the fermentation and oxidation losses. The high DM losses from the large silos,

associated with the high ethanol values suggest that there may have been a yeast -type fermentation in these silages but why this was inhibited in the laboratory tower silos is not known. The values for CO_2 production from the laboratory tower silages confirm the findings of Fyrileiv (Saue and Breirem, 1969) that formic acid inhibits CO_2 production during ensilage.

In experiment F1, the addition of formic acid at the recommended level slowed down the fermentation but did not reduce DM losses from the silages. There was little evidence that the treated material was more stable than, or superior in quality to, the better of the control silages.

VI a. (5) EXPERIMENT F2 - RESULTS.

The following fresh weights of grass were ensiled with the given treatments:-

Silo A - 1261 kg - Control

Silo B - 1261 kg - Control

Silo C - 1382 kg - Treated with 0.34 per cent formic acid

Silo D - 1382 kg - Treated with 0.34 per cent formic acid

Test-tube silos and laboratory tower silos were filled with similar material.

VI a. (5) 1 Volume changes.

Herbage in the control silos was tramped to a volume of $3.02m^3$ before plastic sheeting and stones were placed on the surface. Similar tramping to that in silos A and B resulted in a smaller initial volume ($2.20m^3$) in silos C and D. By the third day, the volumes of the control and treated materials had decreased to $1.56m^3$ and $1.47m^3$ respectively, and at the end of the ensiling period they were $1.26m^3$ and $1.14m^3$.

VI a. (5) 2 Temperature changes.

The highest mean temperature ($14^{\circ}C$) in the control silages was recorded on the second, fourth and fifth days. By the ninth day, the mass had reached $13^{\circ}C$, ambient temperature, and recordings were not made after the 11th day. Temperatures

in the treated silages remained at, or just below, ambient temperature throughout the 11 days.

VI a. (5) 3 Composition.

Compositions of the grasses and silages are shown in Table 24. The higher WSC content of the treated grasses illustrates again the effect formic acid has in preserving these fermentable carbohydrates. The residual sugars were high in the control silages and exceptionally high in the treated silages. Lactic acid was not detected in the latter. Formic acid values for the treated silages were low and suggest a breakdown of formic acid during ensilage. Ethanol values were again higher for the treated silages. The nitrate-N content of the grasses was exceptionally high and recovery of this nitrate in the silages was unexpected. The control silages had a higher volatile-N content than the treated silages. The pH values of the effluents (Fig. 7) from the control silages fell rapidly during the first ten days and then remained fairly constant (around pH 4.0) throughout the experiment. The pH values of the effluents from the treated silages rose from 3.84 to 4.02 in the first seven days, decreased to 3.88 with the increase in effluent flow and rose again to 4.10 by the 16th day. The final pH value of the effluent was 4.2.

The composition of the effluents is shown in Table 25. The effluents from the treated silages had a high WSC content but no lactic acid was detected in them.

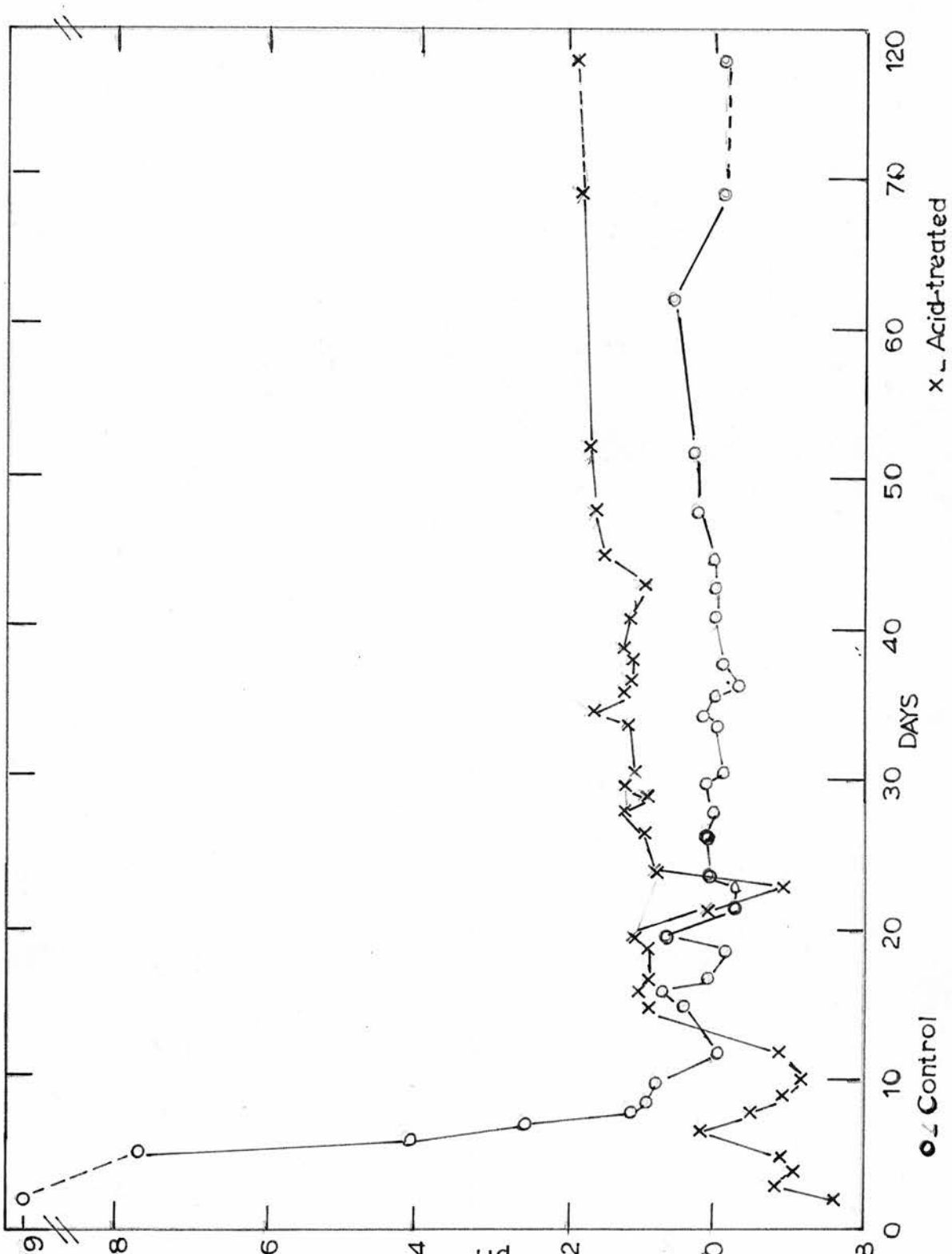


Fig. 7. Changes in effluent pH with time EXPERIMENT F2.

TABLE 24.

Composition of grasses and silages.

	Grasses		Silages			
Treatment	Control	Formic acid -treated (0.34%)	Control		Formic acid -treated (0.34%)	
Silo	AB	CD	A	B	C	D
DM (%)	12.03	11.84	13.06	13.01	15.64	14.70
pH after maceration	5.99	3.81	4.01	4.06	4.26	4.40
Bc (mequiv/100g DM)	25	49	98	98	51	41
Components of DM (%)						
OM	89.8	90.0	90.1	90.0	90.8	89.5
CP	26.1	25.6	24.8	25.0	24.2	23.9
CF	22.2	22.2	22.1	22.5	24.3	24.3
TN	4.18	4.09	3.97	4.00	3.88	3.83
Protein N	3.12	2.98	1.77	1.74	1.92	2.02
NPN	1.06	1.11	2.20	2.26	1.96	1.81
VN	-	-	0.37	0.33	0.18	0.29
VN as % TN	-	-	9.3	8.3	4.6	7.6
Nitrate-N	0.33	0.34	0.30	0.31	0.27	0.25
PN as % TN	74.6	72.9	44.6	43.5	49.5	52.7
WSC	7.5	9.5	2.4	2.2	6.3	6.1
Glucose	1.1	2.0	0.2	0.2	1.2	1.2
Fructose	1.6	2.9	0.1	0.1	1.7	1.3
Xylose	nil	nil	0.1	0.3	0.8	0.7
Galactose	nil	nil	0.3	0.2	0.4	0.4
Arabinose	nil	nil	tr	tr	0.4	0.6
Oligosaccharides (including sucrose)	2.3	1.7	1.3	1.2	0.6	0.6

TABLE 24. (contd.)

	Grasses		Silages			
Treatment	Control	Formic acid -treated (0.34%)	Control		Formic acid -treated (0.34%)	
Silo	AB	CD	A	B	C	D
Fructans	2.0	2.1	nil	nil	0.3	0.3
Mannitol	nil	nil	tr	tr	nil	nil
Cellulose	25.7	26.0	25.7	26.5	27.5	28.2
Lignin	3.6	3.5	3.7	3.6	4.3	4.5
Formic acid	-	2.8	nil	nil	0.4	0.5
Acetic acid	-	-	1.2	1.7	1.2	0.8
Propionic acid	-	-	tr	tr	tr	tr
Butyric acid	-	-	tr	tr	tr	tr
Lactic acid	-	-	8.2	7.4	nil	nil
Succinic acid	-	-	nil	nil	nil	nil
Ethanol	-	-	0.2	0.4	1.5	2.1

TABLE 25.

Composition of effluents.

	From control silages.		From formic acid-treated silages (0.34%)	
Silo	A	B	C	D
DM (%)	4.77	4.73	3.94	3.92
Components of DM (%)				
OM	82.2	82.0	78.8	79.3
N	6.3	6.3	6.3	6.1
Nitrate-N	0.9	0.9	1.2	1.2

TABLE 25. (contd.)

	From control silages.		From formic acid-treated silages (0.34%)	
Silo	A	B	C	D
WSC	9.5	10.2	32.2	30.9
Formic acid	nil	nil	8.8	8.8
Acetic acid	2.9	0.6	0.7	0.9
Propionic acid	tr	tr	nil	nil
Butyric acid	tr	tr	nil	nil
Lactic acid	30.9	14.8	nil	nil
Succinic acid	nil	nil	nil	nil
Ethanol	0.9	0.8	5.4	5.8

VI a. (5) 4. Losses.

The weights (kg) of fresh silage and effluent removed from the silos were:-

		Silage		Effluent
Treatment	Silo	Waste	Good	
Control	A	nil	1074	179
Control	B	nil	1116	139
Formic acid - treated (0.34%)	C	32	843	500
	D	5	923	445

Gaseous losses are shown in Figure 8. Gaseous losses were low from the control and formic acid-treated silages during the early stages of ensilage but later in the ensiling period, the losses were greater from the treated silages. Detailed DM losses are given in Table 26 and the losses of some components of

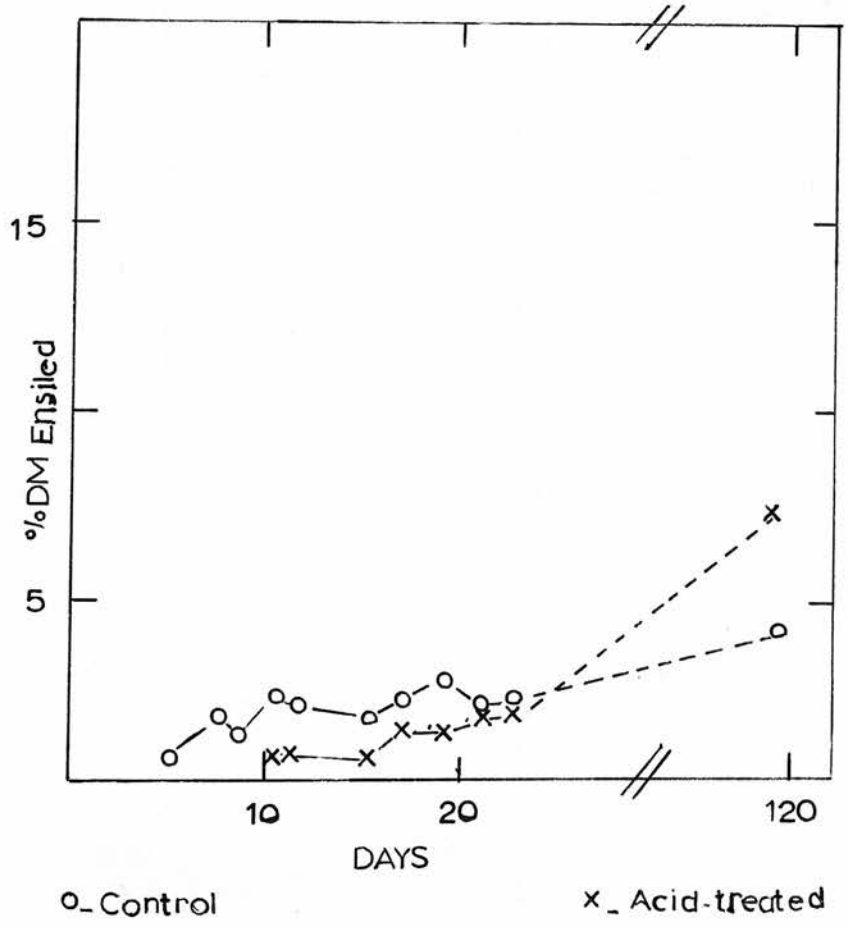


Fig. 8. Gaseous losses as a percentage grass DM ensiled

EXPERIMENT F2.

DM are given in Table 27. Losses of DM from the treated herbages were higher than from the controls, the effluent differences being particularly large. Losses of WSC and formic acid in the effluents from the treated materials were high.

TABLE 26.

Percentage DM losses during ensilage.

Treatment	Control		Formic acid-treated (0.34%)	
Silo	A	B	C	D
Fermentation and oxidation	1.8	nil	4.4	5.9
Effluent	5.7	4.4	12.0	10.7
Waste	nil	nil	3.0	0.5
Total edible loss	7.5	4.4	19.4	17.1

TABLE 27.

Percentage losses of DM components.

Treatment	Control				Formic acid-treated (0.34%)			
Silo	A		B		C		D	
	Total	Effluent	Total	Effluent	Total	Effluent	Total	Effluent
OM	7.3	5.1	4.0	4.0	15.8	10.5	17.1	9.4
N	12.1	8.6	8.3	6.6	20.8	18.7	22.0	16.0
Nitrate-N	16.0	1.6	10.2	1.2	33.2	4.1	38.7	3.8
WSC	71.1	7.0	71.9	6.1	44.5	41.3	46.5	34.8
Formic acid	-	-	-	-	88.1	37.6	85.3	33.3
Cellulose	7.4	-	1.5	-	11.7	-	9.6	-

VI a. (5) 5. Laboratory silos.

The pH values and WSC contents of the silages from the test-tube silos are given in Figures 9 and 10 respectively. The WSC of the control materials followed a similar pattern to those in experiment F1, but in the treated silages the WSC content remained high until the 37th day but had fallen to less than 2 per cent of DM by the 142nd day.

The results of a detailed analysis of the WSC of the grass in the field, as ensiled, and of the herbage after several days in the silo are given in Table 28.

TABLE 28.

Components of WSC in grasses and silages (%DM)

	Control			Formic acid-treated (0.34%)	
	As cut	As ensiled	After 4 days	As ensiled	After 8 days
Glucose	1.0	1.1	0.5	1.7	4.4
Fructose	1.7	1.6	2.7	2.3	5.0
Xylose	nil	nil	0.6	nil	0.5
Galactose	nil	nil	0.2	nil	1.0
Arabinose	nil	nil	0.1	nil	0.8
Oligosaccharides (including sucrose)	3.0	1.7	0.8	1.8	1.4
Fructans	2.8	1.9	0.5	2.2	0.1
Mannitol	nil	nil	1.3	nil	nil

The addition of formic acid conserved WSC between the field and the silo. The inhibition of the bacteria in the treated silages is indicated by the high WSC content and absence of mannitol after eight days. The presence of galactose, arabinose and xylose in the control and treated silages after several days confirms that heteropolysaccharides, as well as fructans and sucrose, were

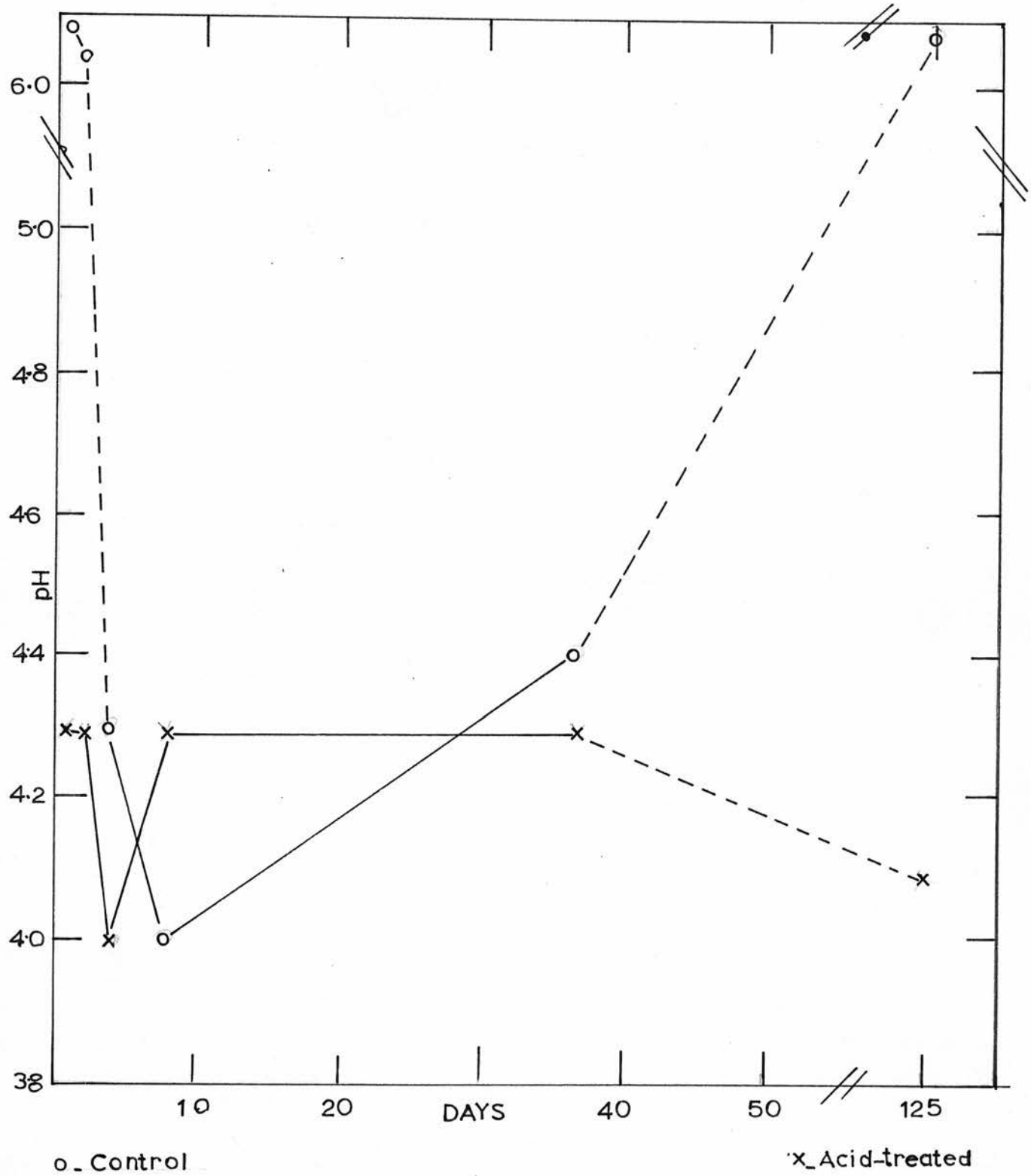


Fig. 9. pH values for laboratory tube silos EXPERIMENT F2.

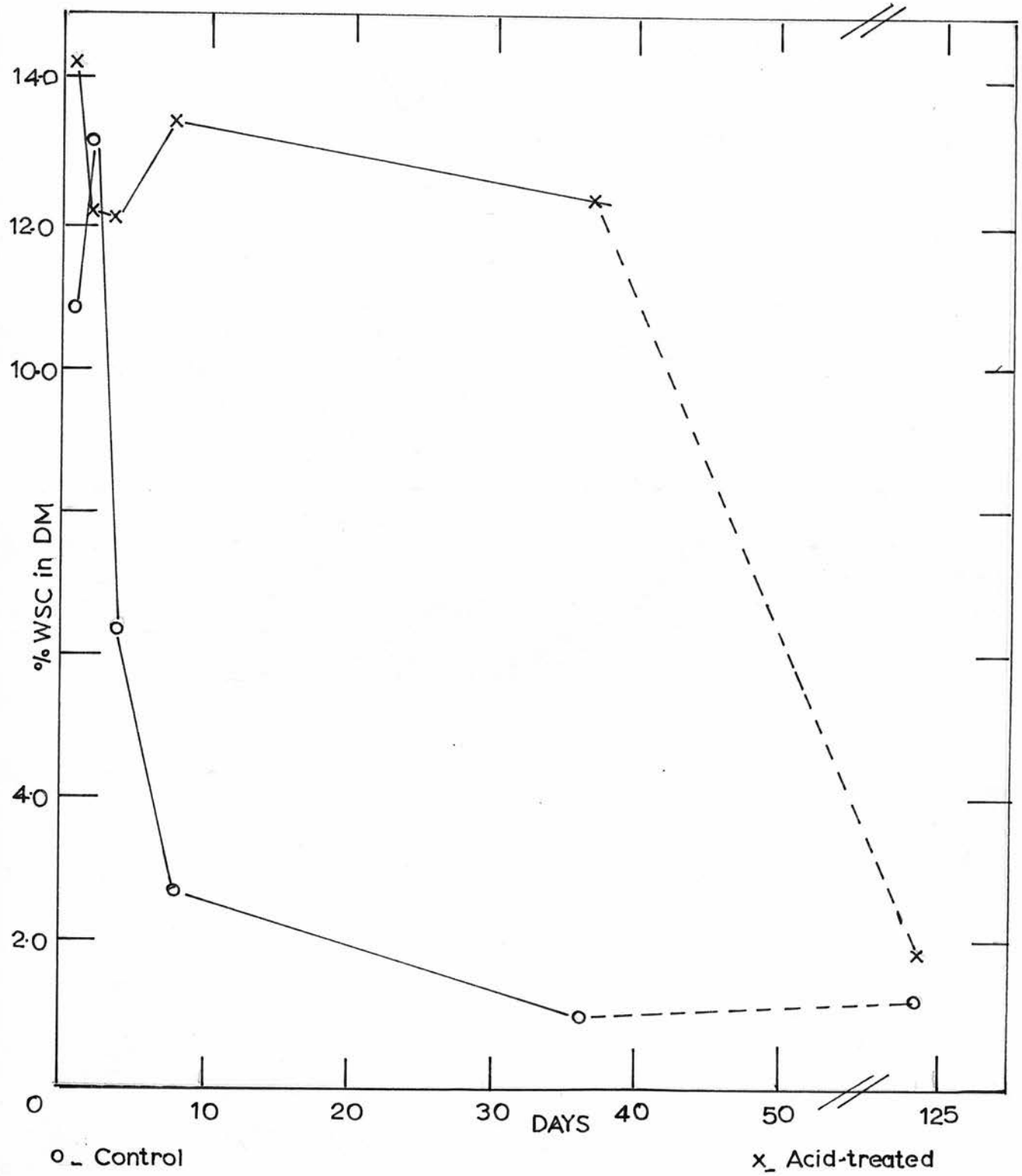


Fig. 10. WSC values for laboratory tube silos EXPERIMENT F2.

hydrolysed during the early stages of ensilage.

The results of the analysis of the silages from the laboratory tower silos (mean of two) and the DM losses and CO_2 production during ensilage are given in Table 29.

TABLE 29.

Analysis of silages and losses of DM from the laboratory tower silos.

Treatment	Control	Formic acid-treated (0.34%).
DM(%)	11.5	11.5
pH	4.62	3.89
Components of DM(%)		
WSC	0.7	4.3
Formic acid	nil	1.1
Acetic acid	6.7	1.1
Propionic acid	tr	nil
Butyric acid	tr	nil
Lactic acid	9.9	6.5
Succinic acid	nil	nil
Ethanol	1.2	1.8
Losses		
Fermentation and oxidation(%)	3.3	4.9
CO_2 absorbed (% DM ensiled)	3.0	1.5

The level of fermentation acids was higher in the control silages than in the treated silages although DM losses from the latter were slightly higher. Less CO_2 was produced in the treated silages but ethanol values were higher.

VI a. (6) DISCUSSION.

The grass ensiled in experiment F2 was similar in composition to that used in experiment F1, having a high CP/WSC ratio and a high nitrate-N content but with a lower buffering capacity. The combination of the latter with the lower DM content resulted in acid application lowering the pH value of the grass to 3.81, lower than might have been expected from the previous experiment. In the large metal silos, this rate of application (0.34 per cent) was sufficient to inhibit the lactic acid bacteria completely, no lactic acid being detected in either the silages or the effluents from silos C and D. The pH value of the effluents from the control silages fell to below pH 4.0 and the silages had high lactic acid and low acetic acid contents. Less proteolysis occured in the treated silages and formic acid had an inhibiting effect on the proteolytic clostridia. The nitrate -N contents of the silages were high, only 10-15 per cent having been broken down in the control silages and 30-35 per cent in the treated silages. The very high DM losses from the treated silages were due mainly to the high effluent production. This level of application of formic acid appeared to affect the structure of the grass, causing it to compact more readily and release more effluent. Losses due to surface waste and fermentation and oxidation were also higher from the treated silages. The ethanol contents of the treated silages in the absence of lactic acid suggest that yeasts may have been active in them.

The fermentation patterns of both the control and treated materials were different in the laboratory silos from those found in the silages from the metal tower silos. The pH value of the control material in the test-tube silos fell rapidly to pH 4.3 and continued to fall below pH 4.0 but at the later stages of ensilage the pH value rose again. It is probable that the degradation of the material in the test-tube silo opened after 142 days was caused by a faulty mercury seal. Although lactic acid contents were high in the silages from the laboratory tower silos, acetic acid levels were high resulting in higher

pH values than those of the control silages from the metal tower silos. There was also more ethanol in the laboratory silages. Although lactic acid bacteria were not active in the treated silages after 37 days, the lower pH value and lower WSC content suggested that they had become active at a later stage. This was confirmed by the presence of lactic acid in the silages from the laboratory tower silos.

The ability of the lactic acid bacteria to overcome the inhibiting effect of the formic acid in the one situation and not in the other may be accounted for by the difference in ambient temperatures. Whereas the laboratory silos were held at approximately 16° - 20°C, the metal tower silos were situated in a barn with the ambient temperature approaching zero on occasions during the winter months. According to Lanigan (1965) the optimum temperature for Lactobacillus plantarum is 30°- 35°C and below this they are much less active.

In experiment F2 the rate of application of formic acid, 1.5 times the recommended level, was sufficient to lower the pH value of this grass to 3.81 and to inhibit lactic acid production completely in the metal tower silos. This did not, however, result in lower fermentation and oxidation losses than from the control silages and it increased the effluent DM loss two-fold.

VI a. (7) EXPERIMENT F3 - RESULTS.

The following fresh weights of grass were ensiled with the given treatments:-

Silo A - 1028 kg - Control

Silo B - 1028 kg - Control

Silo C - 1028 kg - Treated with 0.5l per cent formic acid

Silo D - 1028 kg - Treated with 0.5l per cent formic acid

Test-tube silos were filled with similar material.

VI a. (7) 1. Volume changes.

The initial volumes of the control and treated herbage were 2.57m^3 and 1.70m^3, respectively. The volume of the control material fell rapidly and by the fourth day the corresponding volumes were 1.70m^3 and 1.47m^3, respectively. The silage levels fell throughout the ensiling period and the final volumes were similar (0.83m^3).

VI a. (7) 2. Temperature changes.

The maximum mean temperature (29°C) of the control silages was recorded on the fourth day and had fallen to 21°C by the tenth day. The temperature of the treated silages fell during the first few days to 13°C and then rose, at first slowly, and later rapidly to a maximum value of 33°C on the 21st day.

VI a. (7) 3. Composition.

The composition of the grasses and edible silages are given in Table 30. A separate analysis of each grass was made and the high level of soil contamination is confirmed by the high ash figures of the grass and silages. The edible silages only were analysed for WSC and organic acids. The acid-treated silages contained some residual WSC and lactic acid in this material. Lactic acid, although present in the effluent from the control silages, was no longer present in the silages at the end of the ensiling period.

The pH values (Fig.11) of the control effluents fell rapidly during the first few days and remained below 4.20 until the 25th day; the values then increased until the 80th day, after which they remained fairly constant. Effluents from the treated silages had an initial pH value of 3.96 which rose to 4.08 on the tenth day. There was then only a slight rise in pH to a maximum value of 4.23. On the 93rd day, free drainage was allowed from the silos resulting in a fall in pH value of effluents C and D. During the last 50 days of ensilage, the pH values of these effluents fell from 4.03 to 3.89.

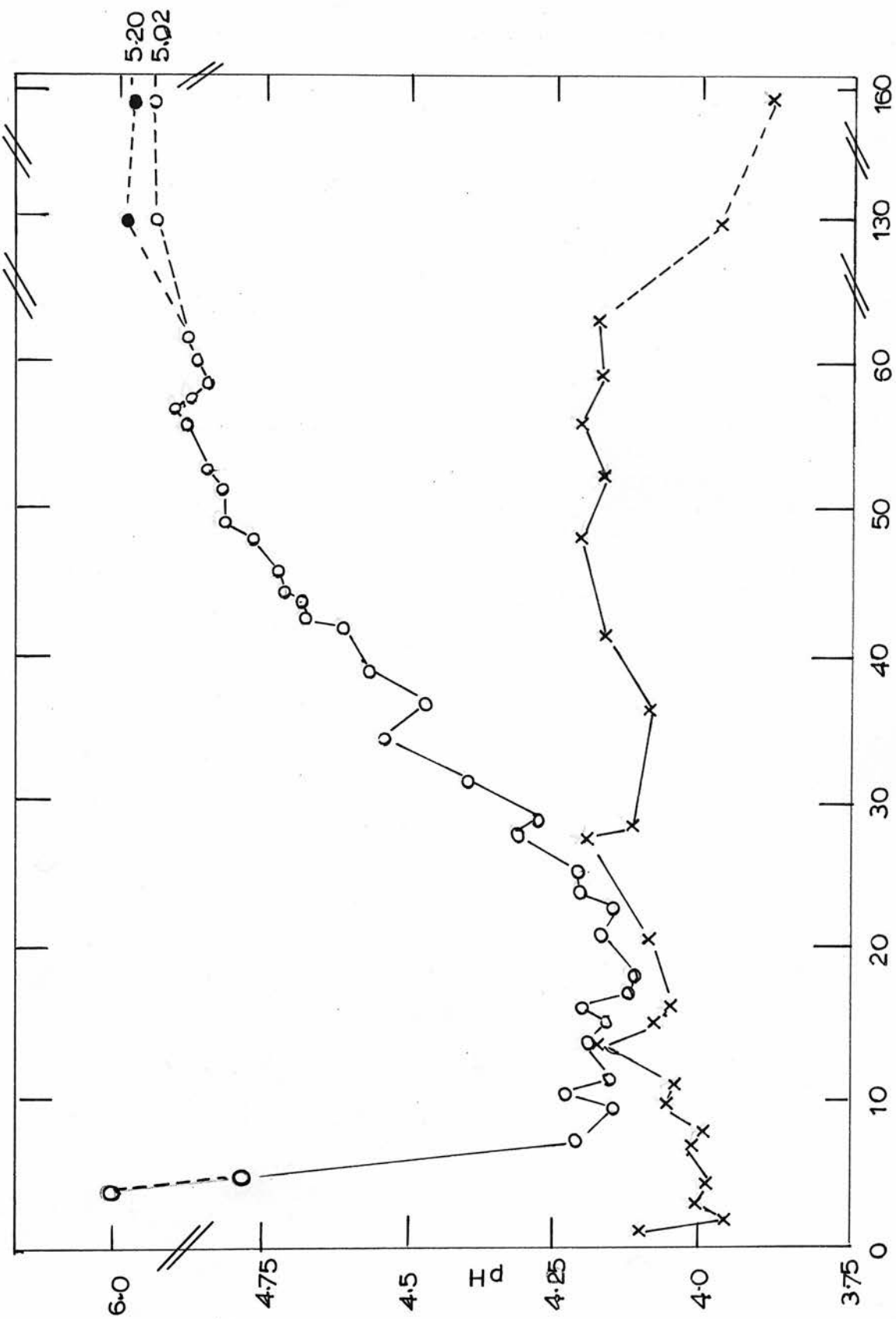


Fig. 11. Changes in effluent pH with time EXPERIMENT F 3.

The composition of the effluents is given in Table 31. The concentration of fermentation acids is higher than the concentration of WSC in the effluents from the control silages. This situation is reversed in the effluents from the treated silages.

TABLE 30.

Composition of grasses and edible silages.

	Grasses				Edible silages			
Treatment	Control		Formic acid -treated (0.51%)		Control		Formic acid -treated (0.51%)	
Silo	A	B	C	D	A	B	C	D
DM (%)	14.64	13.65	16.20	17.30	13.59	16.53	19.84	20.80
pH after maceration	6.01	6.01	4.11	4.11	4.90	4.98	4.19	4.28
Bc (mequiv/100g DM)	41	37	40	44	175	169	94	107
Components of DM (%)								
OM	76.6	81.1	73.9	71.5	73.2	66.9	68.9	68.0
CP	17.4	18.7	18.7	18.3	18.6	17.3	17.4	16.3
CF	20.9	21.6	19.7	19.0	22.9	22.2	20.3	20.7
TN	2.78	2.99	2.99	2.92	2.98	2.77	2.78	2.61
PN	2.16	2.37	2.52	2.45	1.39	1.16	1.43	1.48
NPN	0.62	0.62	0.47	0.47	1.59	1.61	1.35	1.13
VN	0.02	0.02	0.02	0.02	0.76	0.98	0.45	0.37
VN as % TN	0.7	0.7	0.7	0.7	25.5	35.4	15.9	13.7
Nitrate - N	0.11	0.10	0.20	0.20	tr	tr	0.09	0.05
PN as % TN	77.7	79.3	84.3	83.9	46.6	41.9	51.4	56.7
WSC	10.6	9.3	8.8	9.3	0.4	0.2	2.1	2.3
Glucose	2.1	2.5	2.5	2.9	tr	tr	0.2	0.2
Fructose	5.1	4.5	4.2	4.2	nil	nil	0.5	0.5

TABLE 30. (contd.)

	Grasses				Edible silages			
Treatment	Control		Formic acid -treated (0.51%)		Control		Formic acid -treated (0.51%)	
Silo	A	B	C	D	A	B	C	D
Xylose	nil	nil	nil	nil	nil	nil	0.3	0.2
Galactose	nil	nil	nil	nil	tr	tr	0.3	0.3
Arabinose	nil	nil	nil	nil	nil	nil	nil	0.1
Oligosaccharides (including sucrose)	0.6	0.6	0.3	0.3	tr	tr	0.1	0.1
Fructans	1.7	1.0	1.0	1.1	nil	nil	tr	tr
Mannitol	nil	nil	nil	nil	nil	nil	tr	tr
Cellulose	22.1	23.8	22.1	20.8	23.9	23.9	23.5	22.3
Lignin	3.9	3.5	3.7	3.3	4.8	5.4	4.6	5.3
Formic acid	-	-	3.2	3.0	nil	nil	1.3	1.5
Acetic acid	-	-	-	-	8.7	6.3	2.7	3.1
Propionic acid	-	-	-	-	2.0	1.5	0.1	0.3
Butyric acid	-	-	-	-	4.3	3.3	1.1	1.9
Lactic acid	-	-	-	-	nil	nil	4.4	3.9
Succinic acid	-	-	-	-	nil	nil	nil	nil
Ethanol	-	-	-	-	1.2	1.0	0.7	0.4

TABLE 31.

Composition of effluents.

Treatment	From control silages		From formic acid-treated silages (0.51%).	
Silo	A	B	C	D
DM (%)	4.23	4.12	4.71	5.14
Components of DM (% DM)				
OM	76.3	75.5	77.6	78.6
N	5.2	5.9	4.8	4.4
WSC	5.4	3.2	30.7	33.7
Glucose	0.5	0.5	6.1	7.3
Fructose	2.5	1.2	11.9	14.3
Xylose	0.1	tr	1.5	1.7
Galactose	0.6	0.3	5.7	6.2
Arabinose	0.1	tr	1.6	1.6
Oligosaccharides (including sucrose)	0.6	0.3	3.0	1.9
Fructans	0.1	tr	0.3	0.4
Mannitol	1.6	1.1	0.6	2.7
Formic acid	nil	nil	8.5	8.9
Acetic acid	15.3	15.2	1.9	1.5
Propionic acid	3.2	4.4	tr	tr
Butyric acid	4.4	6.9	0.9	0.7
Lactic acid	16.1	8.4	6.9	4.4
Succinic acid	nil	nil	nil	nil
Ethanol	3.9	4.2	3.0	1.9

VI a. (7) 4. Losses.

The weights (kg) of fresh silage and effluent removed from the silos were:-

		Silage		Effluent
Treatment	Silo	Good	Waste	
Control	A	509	207	273
Control	B	373	198	412
Formic acid - treated (0.51%)	C	335	231	430
	D	350	232	412

Gaseous losses are shown in Figure 12. Gaseous losses from the control silages were very high during the first four days and increased gradually to the end of the ensiling period. Losses from the formic acid-treated silages remained low until the tenth day and then followed the same pattern as the losses from the control silages.

Detailed DM losses given in Table 32 show that the waste losses were very high in both control and formic acid-treated materials. The fermentation and oxidation loss from silo D is surprisingly low and could have resulted from a sampling error caused by variable soil content throughout the herbage. Because of this, the OM values in Table 33 are more reliable for comparison purposes than are the DM values.

TABLE 32.

Percentage DM losses during ensilage.

Treatment	Control		Formic acid-treated (0.51%)	
Silo	A	B	C	D
Fermentation and oxidation	18.8	16.4	14.8	1.4

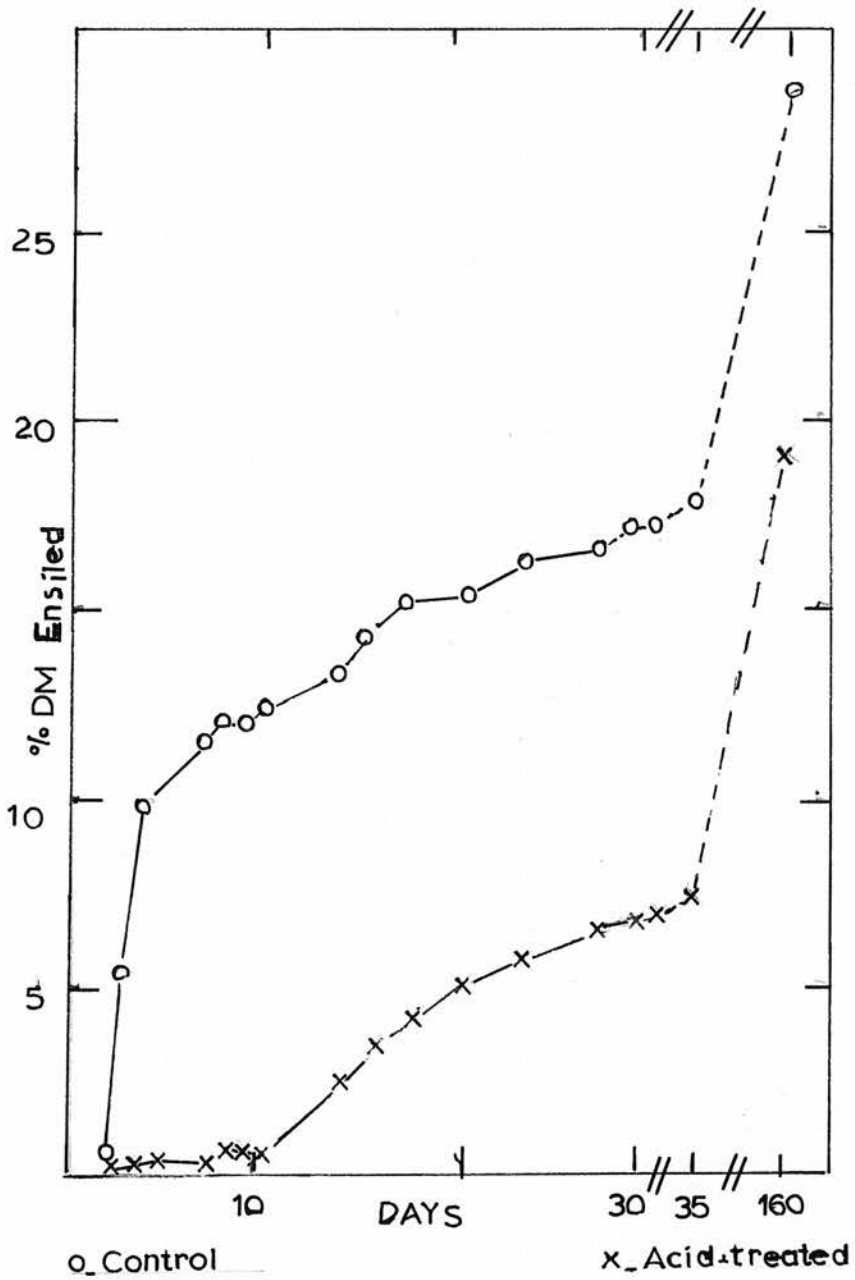


Fig. 12. Gaseous losses as a percentage grass DM ensiled

EXPERIMENT F3.

TABLE 32. (contd.)

Treatment	Control		Formic acid-treated (0.51%)	
Silo	A	B	C	D
Effluent	7.7	12.1	12.1	11.9
Waste	27.5	27.5	33.1	40.5
Total edible loss	54.0	56.0	60.0	53.8

TABLE 33.

Percentage losses of DM components.

Treatment	Control				Formic acid-treated (0.51%)			
Silo	A		B		C		D	
	Total	Effluent	Total	Effluent	Total	Effluent	Total	Effluent
OM	36.5	7.6	42.3	11.3	40.9	12.8	40.4	13.1
TN	26.3	14.3	37.9	23.9	37.6	19.5	45.3	17.7
WSC	98.4	3.9	98.8	4.2	90.7	42.3	90.2	43.1
Formic acid	-	-	-	-	83.5	32.7	79.7	33.5
Cellulose	28.2	-	34.2	-	33.7	-	34.0	-

VI a. (7) 5. Laboratory silos.

The pH values and WSC contents of the silages from the test-tube silos are given in Figures 13 and 14 respectively. The pH value of the control silage fell rapidly and remained low throughout the ensiling period. After a slight rise initially, the pH value of the acid-treated silage fell to a similar value as that of the control silage. The WSC content of the control silage fell rapidly to a low level but the WSC content of the acid-treated silages remained

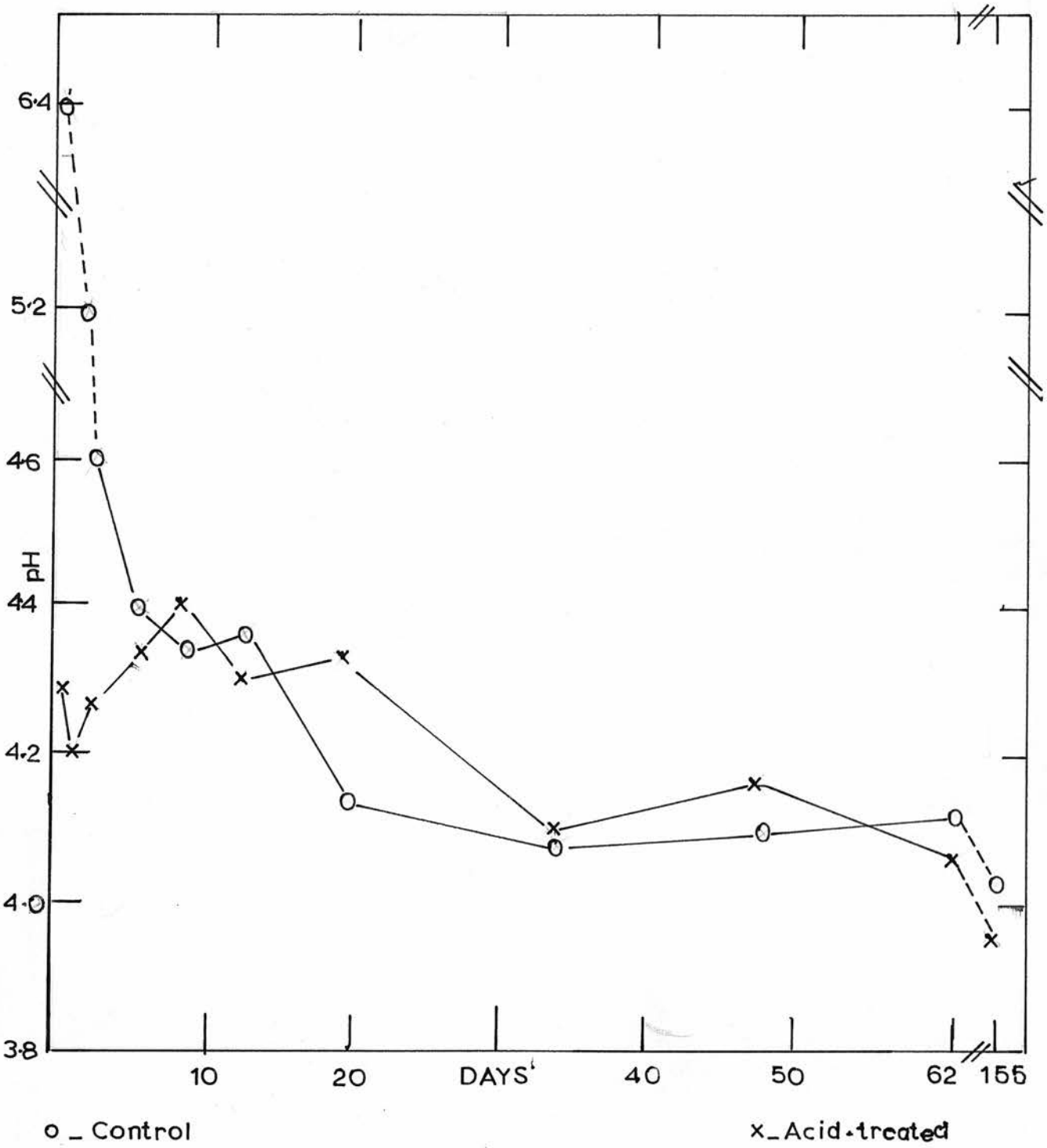


Fig. 13. pH values for laboratory tube silos EXPERIMENT F3.

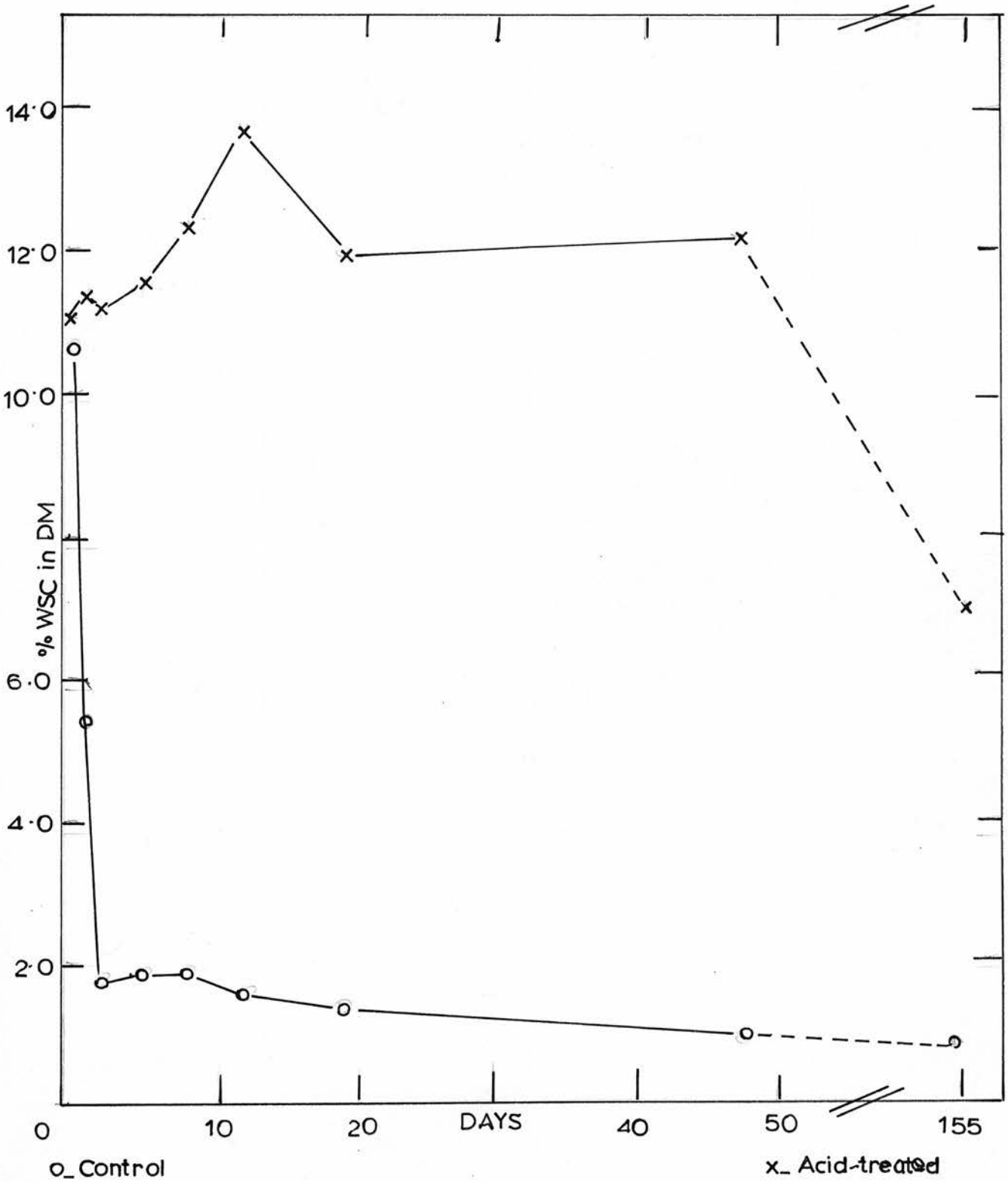


Fig. 14. WSC values for laboratory tube silos EXPERIMENT F3.

high until the 48th day and was still relatively high after 154 days.

The WSC components of the DM of the grasses and silages at the early stages of ensiling are given in Table 34. The acid components of the DM at the later stages of ensiling are given in Table 35.

TABLE 34.

Components of WSC in grasses and test-tube silages (%DM)

Treatment		Control				Formic acid-treated (0.51%)		
	Grass from field	As ensiled	1day	3day	13day	As ensiled	1day	3day	13day
Glucose	3.5	2.0	3.9	0.5	nil	2.2	3.2	3.0	4.6
Fructose	4.2	4.5	4.8	0.9	nil	4.1	5.3	5.2	5.1
Xylose	nil	nil	nil	nil	0.3	nil	nil	tr	0.6
Galactose	nil	nil	0.6	0.2	nil	nil	0.3	0.8	1.2
Arabinose	nil	nil	nil	nil	nil	nil	nil	nil	0.7
Oligosaccharides (including sucrose)	3.8	0.8	0.5	0.3	0.2	1.2	0.7	1.0	0.5
Fructans	1.8	0.9	0.5	0.2	tr	0.7	0.8	0.6	0.2
Mannitol	nil	nil	nil	2.9	3.0	nil	nil	nil	nil

The large discrepancy in WSC content between the grass from the field and the grasses as ensiled in the test-tube silos was in part due to the soil contamination on the forage - harvested grasses. The grass cut with the shears had an ash content of 8.9 per cent of DM whereas the control and treated grasses, as ensiled, had ash contents of 17.3 per cent and 23.0 per cent of DM, respectively. As in the previous experiment, galactose was the first sugar to appear as the result of the hydrolysis of heteropolysaccharides. After 13 days, there was very little sugar left in the control silages and the fructose was replaced by mannitol. In addition to the sugars in the treated grass, xylose, galactose

and arabinose were present in the 13-day silages but mannitol was not detected.

TABLE 35.

Organic acids in test-tube silages during the later stages of ensilage (%DM)

Day	pH		Formic acid		Acetic acid		Lactic acid	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated
34	4.08	4.10	nil	3.2	2.4	0.2	12.2	0.2
62	4.13	4.07	nil	2.4	5.5	0.6	10.6	2.3
154	4.03	4.06	nil	1.9	3.5	1.7	11.6	7.4

There was a marked increase in the concentration of fermentation acids after the 34th day. The formic acid content of the treated silages decreased during the later stages of ensiling.

VI a. (8) DISCUSSION.

Although the rate of application of formic acid (0.51 per cent) was higher in this experiment than in F2, the higher DM content and higher buffering capacity of the Dactylis glomerata prevented the pH value of the grass falling below 4.1. Lactic acid bacteria were not inhibited completely, but there was a delay in the fermentation in both the metal tower silos and the test-tube silos. The pH value of the effluent rose gradually until the 28th day and then decreased. In the test-tube silos, 0.2 per cent lactic acid was detected in the silage analysed on the 34th day. This coincided with a decrease in the formic acid content of the silage. By the end of the ensiling period the lactic acid content of the treated test-tube silage had risen to 7.4 per cent of DM and the pH had fallen to 4.06 but the WSC content was still relatively high (7.0 per cent of DM). This, in conjunction with the low acetic acid value, suggests that homofermentative lactic acid bacteria were mainly responsible for the

conversion of WSC to acid. In the control test-tube silages, the further fall in pH after the 13th day indicates that galactose, arabinose and xylose were used as substrate by the bacteria. The fact that acetic and lactic acids are produced when these sugars are fermented by homofermentative lactic acid bacteria could explain the higher acetic acid content of these silages.

The contamination of the grass with soil, coupled with the extensive losses due to oxidation, make the interpretation of losses difficult in this experiment. Total and effluent losses were similar from all the silages but fermentation and oxidation losses were higher from the control silos. Although aeration caused extensive degradation in all the silages, the treated silage in the lower half of the metal tower silos was superior in quality to the material in the control silos. The formic acid inhibited the proteolytic clostridia and volatile-N contents were much lower. Proteolysis was also less extensive in the treated silages. In the control silages, lactic acid was replaced entirely by butyric acid due to the action of the saccharolytic clostridia. In the treated silages, lactic acid was still present and acetic and butyric acid contents were much lower.

It is clear from experiment F3 that material of high buffering capacity requires a level of formic acid well in excess of the recommended level if the pH value of the grass is to be lowered and deleterious bacteria inhibited completely. If oxidation losses are to be minimised, silos must be carefully sealed as formic acid applied uniformly to the grass, is ineffective in this respect.

VI a.(9) EXPERIMENTS F4 and F5 - RESULTS.

Test-tube silos only were used in these experiments.

VI a. (9) 1 Composition

The grass ensiled in experiment F4 was Dactylis glomerata (DM 21.2 per cent, WSC 11.2 percent of DM) cut from experimental plots with shears. The addition of 0.61 per cent formic acid on the fresh matter reduced the pH value of the

grass from 5.91 to 3.96. Four test-tube silos were filled for each treatment control, treated with 0.61 per cent formic acid and with the same weight of formic acid applied to the surface layer only. Two test-tubes were stored with, and two without mercury seals. The composition of the silages after 28 days (mean of two) is shown in Table 36.

TABLE 36.

Composition of test-tube silages after 28 days.

	Control		Formic acid-treated (0.61 per cent) silages.		Formic acid-treated silages 0.61 per cent on surface.	
	Sealed	Open	Sealed	Open	Sealed	Open
DM	19.7	15.1	21.6	15.7	20.3	15.5
pH	5.5	8.2	4.5	7.9	4.7	8.3
per cent WSC in DM	3.7	0.8	10.7	1.0	4.7	1.2

The pH values of the 'open' silages were high and the WSC contents were low. The pH value of the control silage was surprisingly high, especially as there was 3.7 per cent of WSC in the DM.

The grass used in experiment F5 was Dactylis glomerata, (DM-14.0 per cent) cut with a mower on the farm. It had a high CP content (28.70 per cent of DM) and a very low WSC content (2.6 per cent of DM) and the addition of 0.44 per cent of formic acid on the fresh matter lowered the pH value of the grass from 6.08 to 3.86. The silos were filled as in F4 and the 'open' silages were examined after 34 days. The pH values of the upper and lower halves of the material in each tube are given in Table 37.

TABLE 37

pH values of 'open' silages after 34 days.

		Control	Formic acid-treated (0.44 per cent) silages.	Formic acid-treated silages 0.44 per cent on surface.
pH	Upper	8.2	8.5	8.5
	Lower	6.3	4.5	7.1

The pH values of the upper layers of the 'open' silos were high for all treatments.

The 'sealed' silos were opened after 144 days and the results of analysis are given in Table 38.

TABLE 38.

Composition of sealed silages after 144 days.

	Control	+0.44 per cent formic acid	+0.44 per cent formic acid applied to the surface
DM (%)	13.4	15.5	13.6
pH	6.8	4.9	5.6
Composition of DM (%)			
WSC	0.5	2.7	0.9
Formic acid	nil	2.9	2.2
Acetic acid	9.7	4.2	9.7
Propionic acid	0.9	tr	0.6
Butyric acid	4.2	tr	2.7
Lactic acid	nil	tr	tr
Succinic acid	tr	nil	tr

The acetic acid content of the material treated uniformly with formic acid was lowest after 144 days and only a trace of butyric and lactic acids was detected. There was no lactic acid in the control silage and butyric and acetic acid contents were high. These were also high when the formic acid was applied to the top layer only.

VI a. (10) DISCUSSION

The results of experiments F4 and F5 confirm, on a laboratory scale, the findings of experiment F3 that formic acid, applied uniformly to herbage, does not inhibit the oxidative bacteria. Top spoilage was equally extensive when a high concentration of formic acid was applied to the surface material only.

The high pH values and relatively high residual WSC content of the control silages in experiment F4 suggest that the lactic acid bacteria on the grass from an experimental plot and cut with shears were not sufficient in number to support a normal fermentation. For this reason the experiment was repeated using grass cut with a mower on the farm. As this material was very low in WSC content the 'sealed' silages were examined in more detail after 144 days. As was expected with material of this composition, saccharolytic clostridia were active in the control silages and the concentration of fermentation acids was far in excess of the concentration of WSC in the grass. When formic acid was applied uniformly to the grass the saccharolytic clostridia were inhibited and only a trace of butyric acid was detected in the silages. This would appear to be the result of the concentration of formic acid, rather than the pH value, as the low DM conditions and final pH value of 4.9 would appear to be ideal for their proliferation. When they ensiled lucerne of very low WSC content but with a lower level of formic acid application Wilson and Wilkins (1973) found that saccharolytic clostridia were not inhibited.

The high acetic acid levels are typical of silages prepared from herbages

with a high CP/WSC ratio, such as tropical grasses (Catchpoole, 1965). The source of this acetic acid is not known but it can be produced from the action of proteolytic clostridia on amino acids, and from the fermentation of pentoses by lactic acid bacteria (McDonald and Whittenbury, 1967). Whatever the source, formic acid in this experiment and in the earlier experiments curtailed the production of acetic acid, thereby keeping the pH value down.

In conclusion, the use of formic acid on low DM and low WSC crops does affect the fermentation pattern but in no way reduces oxidative losses. When applied at the recommended level it will reduce losses of WSC due to respiration, inhibit the production of gases at the early stages of ensiling, and keep the temperature of the mass down. The lactic acid fermentation and production of volatile-N will be slowed down but there may be an increase in ethanol production. Applied at a higher rate, it will affect the structure of the grass resulting in a greater loss of DM in the effluent, but it will inhibit deleterious bacteria. At levels higher than those which are considered economically viable, it will inhibit lactic acid bacteria completely and conserve the WSC content of the herbage.

VI b. THE EFFECT OF FORMIC ACID ON THE FERMENTATION OF WILTED GRASS.

VI b. (1) INTRODUCTION.

In the previous section, it was shown that formic acid applied to grasses of low DM changed the fermentation pattern by inhibiting respiration and fermentation initially and, when applied at a sufficiently high rate, conserved some of the WSC present in the grass and discouraged clostridial activity. The advantages obtained from wilting herbage prior to ensiling are stressed by many workers (Brown and Kerr, 1965; Jackson and Anderson, 1968; Murdoch et al, 1955 and Nash, 1959) and include a selective influence on microbial activity (Weise, 1967; Wieringa, 1968), the higher moisture content discouraging clostridial development. Probably the greatest benefit from wilting a crop to 25 -30 per cent DM is the elimination of effluent production and the associated problems of pollution. In this, wilting has a great advantage over an additive applied to the wet crop. Ensiling of wilted crops, however, has its problems, even given good weather conditions. Wilted grass is more difficult to compact and air remains trapped in the silo, resulting in high temperatures due to respiration, and overheated silage of diminished nutritive value. Saue and Breirem, (1969) and Castle and Watson, (1970) reported large differences in the temperature rise during the early stages of ensilage of grasses with and without the addition of formic acid. As the aim in silage making should be to conserve the herbage at a low temperature as close to its original state as possible, a combination of wilting with the addition of formic acid might be acceptable. Both help to minimise fermentation, formic acid reducing respiration and wilting reducing loss of DM in the effluent.

In the experiment discussed in this section (F6), the chemical and microbiological changes occuring during the ensilage of wilted grass, with and without the addition of formic acid, were followed and compared with the chemical changes

occuring in the silage prepared from fresh grass. In addition, test-tube silos were filled with fresh material, fresh material treated with formic acid, wilted material and wilted material treated with formic acid and chemical changes were examined throughout the ensiling period.

VI b. (2) EXPERIMENT F6 - EXPERIMENTAL.

The main silo unit used in this experiment was the same as that used in experiments F1, F2 and F3 and described in the section on Techniques. Lolium perenne was cut with a mower on 8 June, 1970 at 08.00h and wilted for 31h in the field before being lifted with a flail-type forage harvester. Untreated wilted grass was ensiled in silos A and B. The other two metal silos C and D were filled with similar wilted herbage treated with formic acid in the form of ADD-F. The ADD-F was applied to the grass at the rate of 0.39 per cent (equivalent to 0.33 per cent pure formic acid) from a polyethylene container attached to the forage harvester as described earlier.

The ensiled herbage was covered with polyethylene sheeting and after 48h delay was consolidated with stone blocks corresponding to a surface pressure of $27 g/cm^2$. Assessment of true losses and surface waste measurements were made using the bag and marker technique already described. Temperature measurements from eight thermocouples buried at different levels in each silo were recorded daily.

On 8 June, 1970, two reinforced 2000 kg capacity PVC silos (E and F), similar to those used in experiments 01 and 02 were filled with fresh herbage taken from the same field as that used in the wilting experiment. Only chemical changes were studied in these silos.

Silos A, B, C, D, E and F were opened 149, 179, 156, 179, 179 and 114 days after filling, respectively.

Methods of sampling, and chemical and microbiological analysis of grasses

and silages were similar to those described earlier.

In addition to the tower and plastic silos, 52 laboratory test-tube silos (capacity 80g) were filled with herbages similar to those used in the main experiment. An additional treatment was fresh grass plus formic acid, applied at the rate of 0.23 per cent fresh herbage. The laboratory silos, 13 for each of the four treatments, were fitted with mercury seals and duplicates were opened at intervals throughout the ensiling period. Ethanol, WSC components, fermentation acids and pH values are reported for these samples.

VI b. (3) RESULTS.

The following fresh weights of wilted grass were ensiled with the given treatments:-

Silo A - 869kg- Control

Silo B - 869kg- Control

Silo C - 782kg- Treated with 0.33 per cent formic acid

Silo D - 782kg- Treated with 0.33 per cent formic acid

The fresh grass ensiled in silos E and F was not weighed.

VI b. (3) 1 Volume changes.

The initial volumes occupied by the wilted grass in the four metal silos were related to the weight of fresh matter ensiled and the differences remained constant throughout the ensiling period with the silages occupying approximately 70 per cent of the volume occupied by the grass.

VI b. (3) 2 Temperature changes.

The temperature changes in the four silos are shown in Figure 15, each point on the graph representing the mean of eight thermocouple recordings. The temperatures followed a similar pattern i.e. an initial rise followed by a decline after consolidation of the herbage on the third day. Temperatures recorded in the formic acid-treated material were consistently lower than those

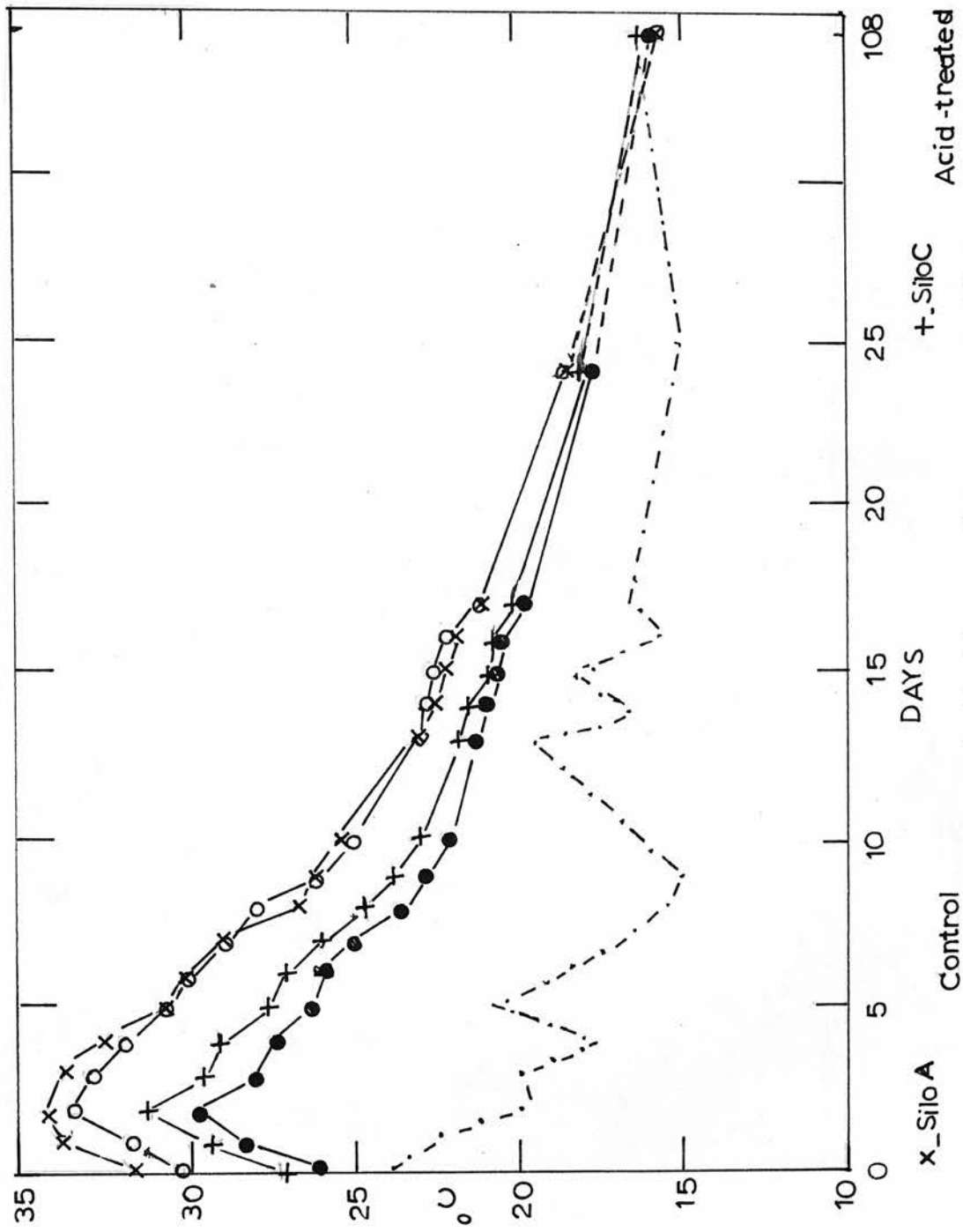


Fig. 15. Temperature changes with time EXPERIMENT F6.

obtained in the untreated material, although the maximum temperatures recorded near the surface of the herbage after 48h were 40°C, 41°C, 44°C and 41°C in silos A, B, C and D respectively. Treatment differences were obvious in the material surrounding the thermocouples placed near the base of the silos. Maximum temperatures recorded in the bottom layers were 31°C, 29°C, 27°C and 26°C in silos A, B, C and D respectively.

VI b. (3) 3 Composition.

The composition of the grasses and silages is shown in Table 39. The DM content of the acid-treated wilted grass (36 per cent) was slightly higher than that of the untreated material (32.3 per cent). The main differences in the DM components are seen in the carbohydrate and organic acid fractions, the WSC percentages being higher and the acetic and lactic acid percentages lower in the formic acid treated-silages compared with the untreated materials. These differences in acid content are also reflected in the pH values of the silages. The unwilted silages are typical of such material, being of low pH value (3.94) and relatively high lactic acid content (10.7 per cent) and containing little residual WSC (1.2 per cent).

TABLE 39.

Composition of grasses and silages.

	Grass			Silage					
	Fresh	Wilted	Wilted and formic	A (W)*	B (W)	C (WF)	D (WF)	E (U)	F (U)
DM (%)	17.75	32.33	36.00	30.82	30.90	32.88	34.49	18.04	18.68
pH after maceration	6.08	6.21	4.93	4.18	4.29	4.39	4.45	3.94	3.94
Bc (mequiv/100g DM)	35	32	35	89	98	54	62	137	112

TABLE 39. (contd.)

	Grass			Silage					
	Fresh	Wilted	Wilted and formic	A (W)*	B (W)	C (WF)	D (WF)	E (U)	F (U)
Components of DM (%)									
OM	93.0	92.8	93.1	92.9	92.0	92.7	92.5	93.1	93.2
CP	14.2	13.4	14.4	14.3	14.0	15.1	15.0	14.6	14.4
CF	26.5	26.9	25.7	28.7	29.3	28.0	29.5	31.0	29.8
TN	2.27	2.15	2.31	2.28	2.24	2.42	2.40	2.34	2.30
PN	1.84	1.47	1.58	0.66	0.47	0.76	0.63	0.36	0.54
NPN	0.43	0.68	0.73	1.62	1.77	1.66	1.77	1.98	1.76
VN	-	-	-	0.19	0.19	0.16	0.16	0.20	0.18
VN as % TN	-	-	-	8.3	8.3	6.8	6.7	8.5	7.8
WSC	17.7	16.8	18.5	4.75	4.56	15.1	15.4	1.44	0.99
Glucose	4.4	3.1	4.8	1.6	1.5	3.2	4.0	0.3	0.2
Fructose	2.9	3.4	3.9	1.4	0.9	7.5	7.4	0.3	0.3
Xylose	-	-	-	tr	0.1	0.2	0.2	0.1	tr
Galactose	-	-	-	0.9	0.9	1.5	1.2	0.3	0.1
Arabinose	-	-	-	tr	0.2	0.3	0.2	tr	tr
Oligosaccharides (including sucrose)	4.0	5.0	5.0	0.7	0.8	2.2	2.2	0.4	0.3
Fructans	6.4	5.3	4.8	0.1	0.1	0.1	0.1	0.1	0.1
Mannitol	-	-	-	3.6	3.7	1.9	1.1	4.3	4.1
Cellulose	27.5	26.5	26.0	28.6	28.8	28.6	29.5	30.1	30.2
Lignin	4.0	4.4	4.3	4.4	4.4	4.4	4.2	4.7	4.1
Formic acid	-	-	0.92	nil	nil	0.5	0.6	0.1	tr
Acetic acid	-	-	-	2.4	3.0	0.8	0.9	3.2	3.6

TABLE 39. (contd.)

	Grass			Silage					
	Fresh	Wilted	Wilted and formic	A (W)*	B (W)	C (WF)	D (WF)	E (U)	F (U)
Propionic acid	-	-	-	0.03	0.05	0.08	0.05	0.18	0.17
Butyric acid	-	-	-	0.06	0.12	0.06	0.10	0.17	0.14
Lactic acid	-	-	-	5.9	8.2	4.3	4.8	11.1	10.2
Succinic acid	-	-	-	nil	nil	nil	nil	tr	tr
Ethanol	-	-	-	0.64	0.61	0.61	0.68	1.2	1.2

* W= Wilted; F = formic acid-treated; U = unwilted.

VI b. (3) 4 Microbiological assay.

Core samples were taken from side ports in silos A and C after one, two, three and four days and results of microbial counts for these together with counts on original grass samples and final silages are given, using five different media, in Table 40. Although total counts of micro-organisms were consistently lower in the formic acid-treated silages there was no evidence from the Tween acetate agar counts, with the possible exception of the samples obtained after two days, to indicate that the activities of lactic acid bacteria were lower in the formic acid-treated material than in the untreated herbage. Microbial proteolytic and lactate fermenting activities were negligible.

TABLE 40.

Microbiological assay

Sample	Bacterial count (no. of organisms/10g of fresh material) YEA+	TAA	MEA	PC	LF	pH	Per cent Ethanol in DM	Per cent WSC in DM
Grass								
Uncut, 8 June, 08.00h	1.2×10^4	1.0×10^3	$< 10^2$	10^4	$< 10^2$	6.20	-	19.3
Forage harvested grass, 8 June, 08.05h	2.2×10^7	3.7×10^3	3.0×10^2	10^4	$< 10^2$	-	-	-
Wilted grass, 9 June, 09.00h	2.9×10^7	1.7×10^5	3.0×10^2	10^4	$< 10^2$	-	-	-
Wilted grass, 9 June, 15.00h	4.5×10^7	1.1×10^6	$< 10^2$	10^4	$< 10^2$	-	-	-
Forage harvested wilted grass, 9 June, 16.00h	4.6×10^7	7.2×10^6	$< 10^2$	10^4	$< 10^2$	6.21	-	16.8
Silage*								
After 1 day Silo A	6.5×10^9	1.0×10^8	3.7×10^3	10^5	$< 10^2$	5.91		17.9
After 1 day Silo C	5.3×10^6	1.0×10^8	$< 10^2$	10^4	$< 10^2$	5.42	-	20.9
After 2 days Silo A	2.8×10^9	1.3×10^8	2.6×10^2	10^5	$< 10^2$	5.00	-	10.3
After 2 days Silo C	4.9×10^6	1.0×10^6	1.0×10^3	10^5	$< 10^2$	5.11	-	20.0
After 3 days Silo A	1.7×10^{10}	7.3×10^7	4.6×10^2	10^6	$< 10^2$	4.85	0.5	-
After 3 days Silo C	3.1×10^7	1.4×10^7	2.9×10^3	10^4	$< 10^2$	5.44	0.6	-
After 4 days Silo A	5.2×10^9	8.5×10^9	4.5×10^4	10^4	$< 10^2$	4.80	0.5	9.8
After 4 days Silo C	1.1×10^9	1.1×10^9	1.0×10^5	10^4	$< 10^2$	5.24	0.3	22.2

TABLE 40. (contd.)

Sample	Bacterial count (no. of organisms/10g of fresh material) YEA+	TAA	MEA	PC	LF	pH	Per cent Ethanol in DM	Per cent WSC in DM
Silage*								
After 149 days Silo A	2.6×10^7	1.2×10^7	$<10^2$	$<10^2$	$<10^2$	4.18	0.6	4.8
After 156 days Silo C	2.5×10^6	1.6×10^8	6.4×10^4	$<10^2$	$<10^2$	4.39	0.6	15.1

* Silage samples, except final ones, taken from the side port using a corer.

+ YEA - Yeast extract agar

TAA - Tween acetate agar

MEA - Malt extract agar

PC - Proteolytic clostridia

LF - Lactate fermenters

VI b. (3) 5 Losses.

The weights (kg) of silage removed from the silos were:-

Treatment	Silo	Silage	
		Waste	Edible
Wilted	A	63	794
Wilted	B	85	773
Wilted + 0.33 per cent	C	116	652
formic-acid	D	81	694

Detailed DM losses are given in Table 41 and losses of some individual component of DM in Table 42.

TABLE 41.

DM losses during ensilage (per cent DM ensiled).

Treatment	Wilted		Wilted + 0.33 per cent formic acid	
Silo	A	B	C	D
Fermentation and oxidation in edible silage.	3.0	3.0	8.0	7.6
Waste	10.4	12.4	15.6	11.1
Total edible loss	13.4	15.4	23.6	18.7

TABLE 42.

Percentage losses of DM components in edible material.

Treatment	Wilted		Wilted + 0.33 per cent formic acid	
Silo	A	B	C	D
OM	3.0	3.8	8.4	8.9
TN	+3.0	+1.1	3.6	4.2

TABLE 42. (contd.)

Treatment	Wilted		Wilted + 0.33 per cent formic acid	
Silo	A	B	C	D
WSC	72.5	73.6	25.2	22.6
Formic acid	-	-	50.2	40.0
Cellulose	+4.6	+5.5	+1.1	+4.8

The fermentation and oxidation losses were calculated using the bag and marker technique and were higher from the formic acid-treated wilted material than from the wilted silages. Recoveries of WSC were high in the formic acid -treated wilted silages.

VI b. (3) 6 Laboratory test-tube silos.

The composition of the grasses ensiled in the test-tube silos is given in Table 43.

TABLE 43.

Composition of grasses ensiled in test-tube silos.

Treatment	Fresh	Fresh + 0.23 per cent formic acid	Wilted	Wilted + 0.33 per cent formic acid
DM (%)	18.3	18.3	32.2	32.6
pH	6.10	4.50	6.21	5.08
WSC in DM (%)	17.0	17.0	18.5	18.3

The formic acid was applied to the fresh grass at the 0.23 per cent level in the laboratory and to the wilted grass at the 0.33 per cent level in the field.

The analytical results of the laboratory silages are shown in graph form in Figure 16 and the individual results are given in the Appendix 2 (Tables A13 to A15). The pH values of the treated and untreated wilted silages from the test

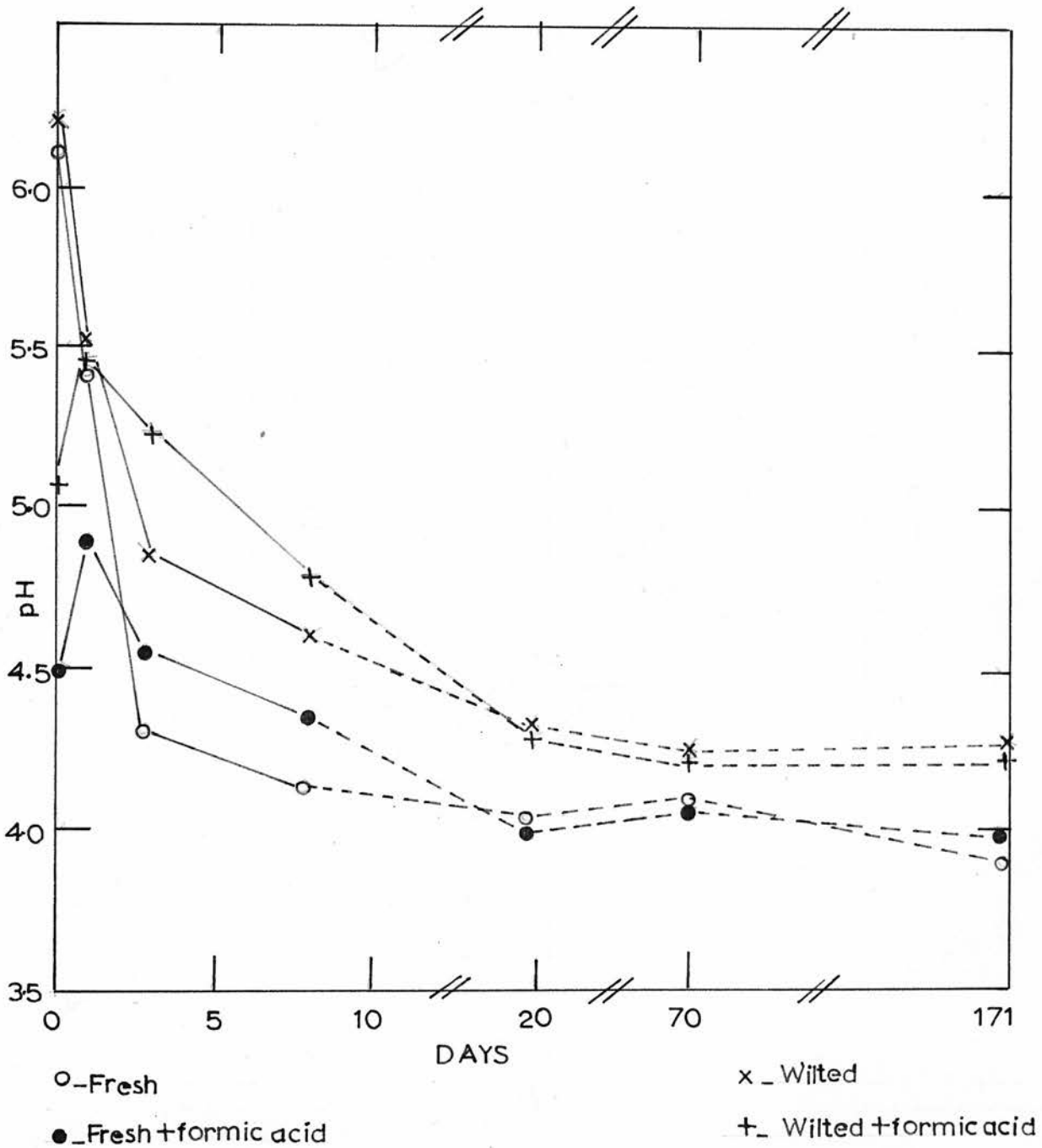


Fig. 16a. pH values for laboratory tube silos EXPERIMENT F6.

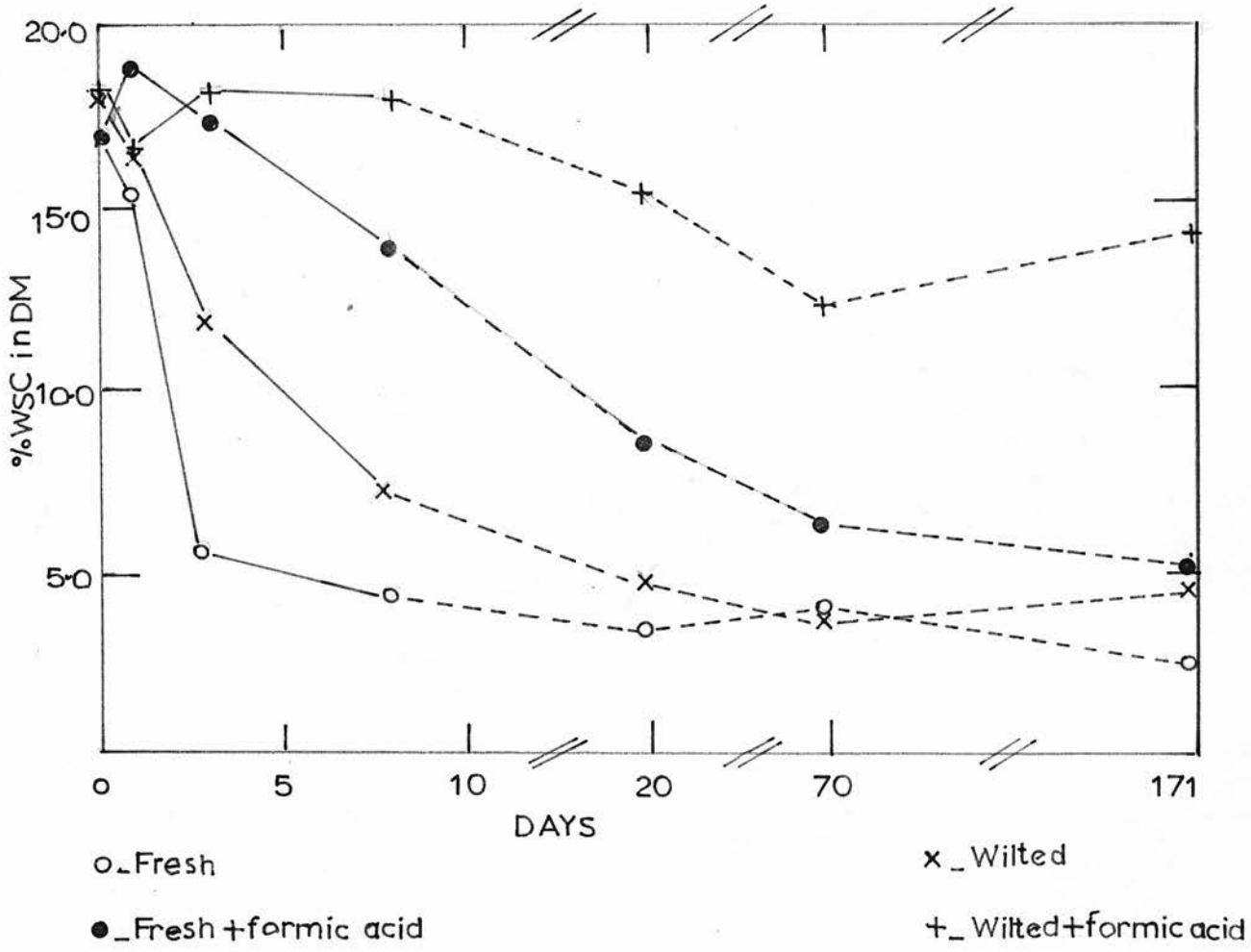


Fig. 16b. WSC values for laboratory tube silos EXPERIMENT F6.

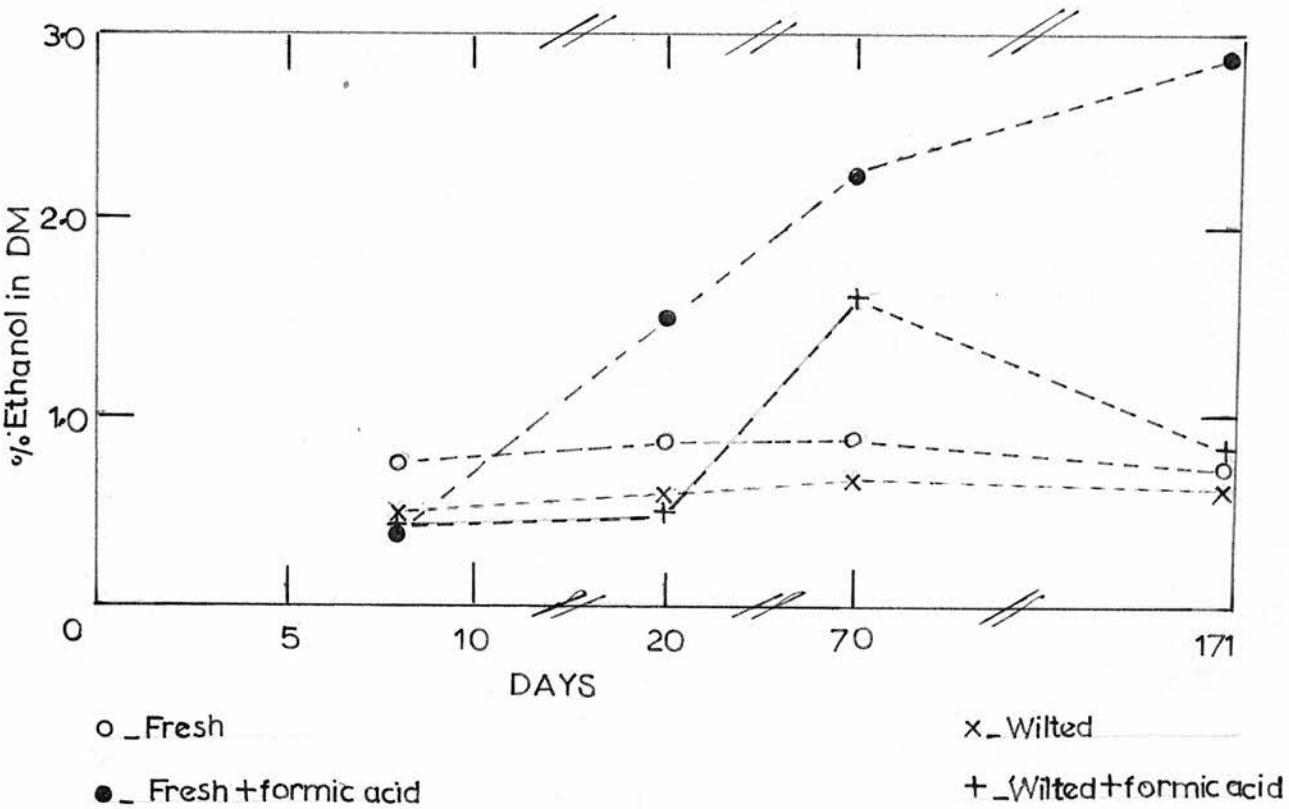


Fig. 16c. Ethanol values for laboratory tube silos EXPERIMENT F6.

-tube silos followed a similar pattern to those of the core samples from the metal tower silos. The pH values of the control and formic acid-treated fresh silages were similar after the 20th day.

The WSC percentages were consistently higher in the formic acid-treated wilted silages than in the untreated silages. Applied to the fresh grass, formic acid had only a slight effect in preserving WSC. The ethanol values for the acid-treated fresh silages were about four times those obtained for the other silages in the final samples.

The WSC components and fermentation acids (mean of two silages) as a percentage of the DM ensiled, are given in Tables 44 and 45 respectively, and a chromatogram showing the changes in WSC components during ensilage is shown in Figure 17.

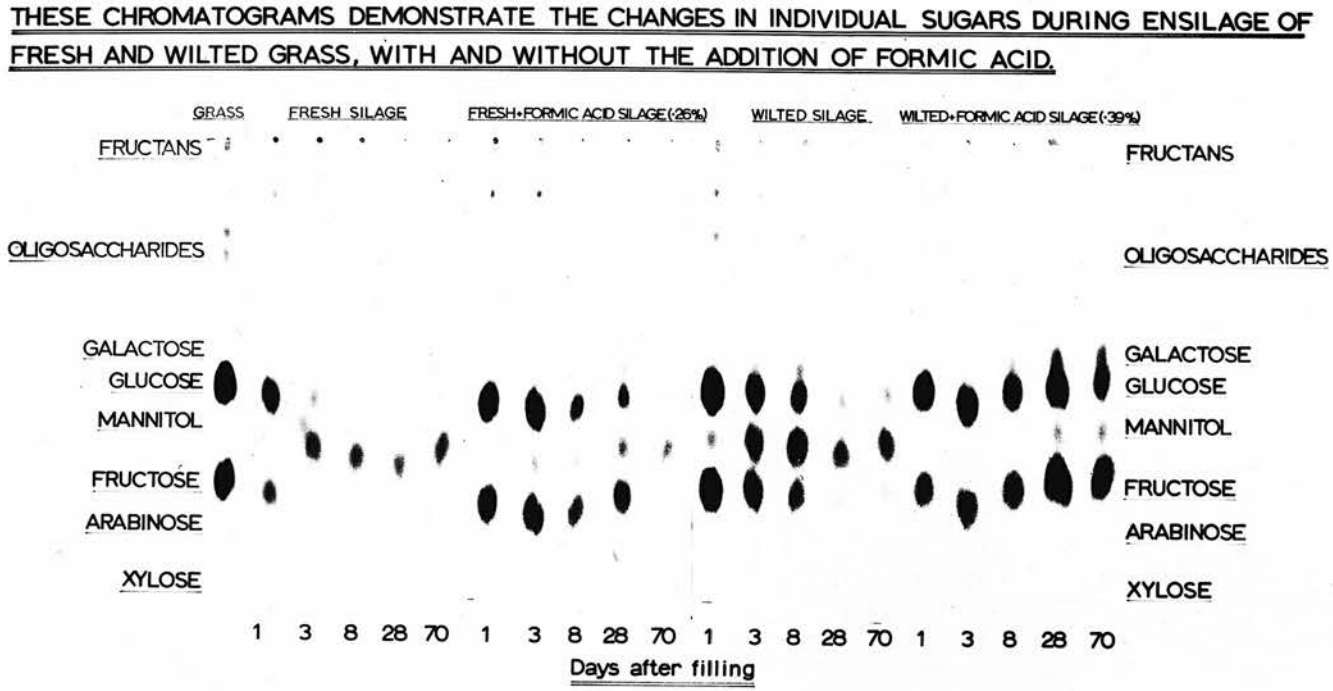


Fig. 17. Changes in WSC components during ensilage EXPERIMENT F 6.

TABLE 44.

WSC components in test-tube silages (per cent DM ensiled)

	FRESH						FRESH + FORMIC ACID						WILTED						WILTED + FORMIC ACID					
DAY	1	3	8	28	70	171	1	3	8	28	70	171	1	3	8	28	70	171	1	3	8	28	70	171
Glucose	4.1	0.8	tr.	tr.	tr.	nil	5.6	4.3	4.6	1.3	0.7	0.9	5.3	2.9	2.2	1.2	0.5	0.4	5.9	5.1	5.4	3.2	3.2	2.5
Fructose	4.6	1.5	1.7	1.2	0.5	0.5	4.0	5.3	5.0	5.0	4.1	2.1	5.0	4.2	2.9	2.1	1.9	1.3	6.2	9.5	9.3	10.2	6.2	7.8
Xylose	tr.	tr.	tr.	tr.	0.5	0.3	nil	tr.	tr.	0.3	0.3	0.6	nil	nil	nil	nil	tr.	0.1	nil	nil	nil	nil	0.2	tr.
Galactose	tr.	0.3	0.4	0.5	0.5	0.2	0.5	0.4	0.5	0.7	0.4	0.6	nil	0.7	0.6	0.4	0.6	0.8	nil	nil	tr.	1.7	1.4	0.9
Arabinose	nil	tr.	tr.	tr.	0.3	0.2	nil	tr.	tr.	0.2	0.2	0.2	nil	nil	nil	nil	tr.	tr.	nil	nil	nil	nil	tr.	tr.
Oligosaccharides (incl. sucrose)	1.2	0.5	0.1	0.3	0.4	0.5	3.1	0.5	0.9	0.6	0.3	0.5	1.7	0.9	0.6	0.3	0.6	0.8	0.3	0.9	0.8	0.7	0.7	2.7
Fructans	5.0	3.1	2.1	0.3	0.2	0.1	4.8	5.7	1.4	0.5	0.1	0.1	3.9	2.7	1.0	0.4	0.1	0.1	3.9	2.3	1.4	0.4	0.3	0.1
Mannitol	0.5	5.6	4.3	6.9	8.6	8.0	nil	1.1	1.7	2.7	3.4	3.6	nil	4.1	3.1	6.0	8.6	5.6	nil	nil	nil	1.2	tr.	tr.

TABLE 45.

Fermentation acids in test-tube silages (Per cent DM ensiled)

	FRESH						FRESH + FORMIC ACID						WILTED						WILTED + FORMIC ACID					
Day	1	3	8	28	70	171	1	3	8	28	70	171	1	3	8	28	70	171	1	3	8	28	70	171
Formic acid	tr.	tr.	tr.	tr.	tr.	tr.	0.8	0.7	0.6	0.6	0.6	0.8	tr.	tr.	tr.	tr.	tr.	tr.	0.5	0.4	0.4	0.4	0.5	0.4
Acetic acid	0.5	1.3	2.1	2.1	2.4	2.2	0.3	0.4	0.7	0.9	1.0	1.6	0.5	1.1	1.6	1.5	2.0	2.2	0.2	0.3	0.3	0.6	0.8	0.9
Propionic acid	0.1	0.1	tr.	tr.	0.1	0.1	tr.	tr.	tr.	tr.	0.1	tr.	tr.	tr.	0.1	tr.	tr.	0.1	tr.	0.1	0.1	0.1	0.1	0.1
Butyric acid	0.1	0.1	0.2	0.2	0.2	0.1	tr.	0.1	tr.	tr.	0.2	0.1	0.1	tr.	0.1	0.1	tr.	0.1	tr.	0.1	0.1	0.1	0.1	0.1
Lactic acid	1.4	6.9	9.2	9.7	9.8	13.8	tr.	2.1	5.5	7.4	7.9	9.1	0.5	4.8	7.0	6.3	6.5	7.4	tr.	1.3	2.8	6.5	7.7	6.9
Succinic acid	0.3	0.1	0.2	0.3	0.1	0.1	0.1	tr.	tr.	tr.	0.1	0.1	0.6	0.9	1.0	0.7	0.7	0.9	tr.	tr.	tr.	tr.	tr.	tr.

The WSC content of the untreated fresh silages decreased rapidly with a concomitant production of fermentation acids, mainly lactic acid. Only a trace of glucose remained in the silages analysed on the eighth day and by the 28th day over 90 per cent of the fructan was hydrolysed. Galactose was the first of the sugars from the breakdown of heteropolysaccharides to be detected in any quantity. A large percentage of the fructose was replaced by mannitol. Although most of the WSC disappeared in the early stages of ensilage, lactic acid bacteria were active at the later stages of ensilage with an increase in lactic acid content after the 70th day.

In the formic acid-treated fresh silages, the inhibiting effect of the acid on the bacteria was seen in a less rapid breakdown of WSC, and a measurable quantity of glucose was present after 171 days. Hydrolysis of fructan was again almost complete by the 28th day but less fructose was converted to mannitol and a larger proportion remained in the silage. Galactose and xylose were also present in slightly higher amounts than in the untreated fresh silage. The inhibition of bacterial activity also resulted in a slower production of fermentation acids with acetic acid and lactic acid remaining lower than in the untreated fresh silages throughout the ensiling period. After 171 days the proportion of acetic to lactic was similar in the silages of both treatments. Values for succinic acid were lower in the formic acid-treated silages.

The inhibiting effect on the bacteria of wilting was seen in a slower breakdown of WSC and less rapid production of fermentation acids in the untreated wilted silages. The rate of hydrolysis of fructan was the same but the rate of disappearance of glucose and fructose lay between that of the fresh and that of formic acid-treated fresh silages. Xylose and arabinose were only present in trace amounts but galactose contents were similar to those found in the formic acid-treated fresh silages. The rate of production of acetic acid was slower

than in the untreated fresh silages but it levelled off at a similar value. Lactic acid contents did not increase much during the later stages of ensilage although there was a decrease in WSC content. Mannitol contents were similar to those of the untreated fresh silages and succinic acid contents were higher.

The combined effects of the addition of formic acid and of wilting are seen in the very slow breakdown of glucose and fructose in the formic acid-treated wilted silages. Fructans were hydrolysed at a similar rate but mannitol production was very low. Galactose was slower to appear but values were slightly higher than in the silages of the other treatments. Arabinose and xylose were only present in trace amounts. In every treatment, oligosaccharides were hydrolysed rapidly but there was an increase in oligosaccharide content towards the end of the ensiling period. This was most obvious in the formic acid-treated wilted silage. Despite the apparent rise in WSC content during the early stages of ensiling in this treatment, lactic acid bacteria were active and the lactic acid content reached a level similar to that of the wilted silage by the 28th day. Acetic acid remained low throughout the ensiling period and succinic acid was only present in trace amounts. Butyric and propionic acid contents were low in all the silages.

VI b. (4) DISCUSSION.

Smith (1962) demonstrated that the availability of inorganic ions to form buffer systems with the weak organic acids produced during ensilage is reduced by wilting the crop (Medicago sativa). In this laboratory, when 0.23 per cent formic acid was added to fresh grasses, Lolium perenne (18.0 per cent DM), Dactylis glomerata (15.6 per cent DM) and Lolium multiflorum (18.2 per cent DM), the pH values of macerates of the treated grasses were 4.59, 4.58 and 4.67 respectively. These grasses were wilted to 32.9, 52.6 and 41.8 per cent DM respectively and the same weight of formic acid was applied to wilted grass

containing the same weight of DM as the fresh grass. The pH values of macerates were Lolium perenne, 4.52, Dactylis glomerata, 4.49 and Lolium multiflorum, 4.70, values not dissimilar from those obtained with fresh grass. A lower level of formic acid application on a DM basis than that required for unwilted grass should have a favourable influence on the fermentation pattern during ensilage as the drier the crop, the less will be the bacterial activity (Gibson and Stirling, 1959) and the higher will be the pH value at which the silage is stable.

In experiment F6, the application of 0.23 per cent on the fresh grass (18.3 per cent DM) lowered the pH value to 4.50, while the application of 0.33 per cent on the wilted grass (36.0 per cent DM) lowered the pH value to 4.93. Wilting had only a slight effect on the buffering capacity, reducing it from 35 to 32 mequiv/100g DM. The wilted grasses were similar in composition and, with the exception of DM andNPN, resembled the fresh grass. The increase in NPN during wilting confirms the results obtained in a previous experiment (McDonald et al, 1968) that proteolytic enzymes become active immediately after harvesting. There was evidence, as in experiments F1 and F2, that formic acid reduces the loss of WSC between the field and the silo by inhibiting respiration.

The mean temperature readings for the material in the four metal silos confirm the findings of other workers (Saue and Breirem, 1969; Castle and Watson, 1970) that formic acid controls the temperature rise in silages. The fact that the temperatures in the surface layers reached the same high levels for both treatments on the third day confirms that the application of formic acid in no way replaces adequate consolidation and efficient sealing of a silo. The temperatures in all the silages decreased after the application of consolidation weights on the third day.

When McDonald et al (1968) wilted grass from 15.9 per cent DM to 30.3 per

cent DM, the fermentation was restricted and fermentation acids were lower and WSC percentages higher than in the fresh silages. The results of Anderson and Jackson (1970) were similar but indicated that a homolactic type of fermentation occurred in the ensilage of the fresh grass/clover mixture, while a heterolactic type of fermentation occurred when similar material was wilted to three different levels. The results of McDonald et al did not support this finding. In the present experiment, wilting again inhibited the production of fermentation acids and WSC contents were higher in the wilted silages than in the fresh silages. There is no evidence from these results that wilting favoured the heterofermentative lactic acid bacteria as the mannitol contents of the fresh silages were relatively high. A similar fermentation pattern is evident in the test-tube silages in which wilting is seen to slow down the lactic acid production. This is claimed by Lanigan (1963) to be due to the reduced availability of the nutrients.

The further inhibition of lactic acid bacteria and the production of a silage of high WSC content was obtained by McDonald et al (1968) when they wilted Lolium multiflorum to 47.0 per cent DM. The addition of 0.33 per cent of formic acid on the wilted material in experiment F6 had a similar effect on the conservation of WSC but more lactic acid was produced and the pH value fell to a lower level than in the high DM silage. Acetic acid and mannitol levels were much lower in the formic acid-treated wilted material than in the untreated wilted silages, suggesting that formic acid had favoured the activity of the homofermentative lactic acid bacteria while inhibiting the heterofermentative lactic acid bacteria. This does not, however, explain the higher fermentation and oxidation losses from the formic acid-treated wilted silages than from the untreated wilted silages. A similar pattern of losses was found in experiments F1 and F2 when fresh grass was treated with formic acid. Beck(1968) claimed that formate, at the lower levels of concentration, leads to a stimulation in growth of

clostridia, coliforms and yeasts. With the exception of the core samples taken on the first day, counts of yeasts and moulds were higher in the formic acid -treated silages. In the earlier work, high ethanol contents in the treated silages supported the theory that the losses were due in part at least to yeast activity. Although ethanol values were again high in the unwilted acid-treated silages from the test-tubes, differences in the ethanol content of the treated and untreated wilted silages were small. The formic acid-treated wilted silages from the test-tubes opened after 70 days did contain more ethanol, however, and this, along with the low acetic acid and mannitol contents, lends support to the hypothesis that this ethanol is a result of yeast activity rather than a by-product of the fermentation of glucose by heterofermentative lactic acid bacteria.

Formic acid had no obvious effect in preventing proteolysis during ensilage although it appeared to have a slight effect in inhibiting deamination. The fermentation in all the silages was dominated by lactic acid bacteria and only small amounts of the products of clostridial activity were present. The micro -biological data obtained for the core samples confirm that proteolytic clostridia and lactate fermenters were relatively inactive in the wilted silages. The lactic acid bacteria increased on the grass during wilting and also immediately after forage harvesting. With the exception of the second day, counts of lactic acid bacteria were similar for both the wilted and formic acid-treated wilted silages.

The analytical results for the silages from the test-tube silos confirm the effect of formic acid, applied at the recommended level, on the fermentation pattern found in the earlier work during the ensilage of fresh grass. The pH and WSC graphs are similar to those of the test-tube silages in experiment F1, with the exceptionof the rise in pH value of the untreated material at the end of the ensiling period in the earlier experiment. The higher WSC/CP ratio of the grass ensiled in experiment F6 ensured a stable silage of high lactic acid

content and low pH value, while in experiment F1, there was an apparent decline in lactic acid content and increase in acetic acid content. In experiment F6, the activity of the lactic acid bacteria in the fresh silage continued throughout the ensiling period, but in the formic acid-treated silages this activity was inhibited resulting in lower levels of lactic acid. Possibly due to the availability of WSC in these silages, ethanol contents were higher than in the formic acid-treated silages in F1.

Only one level of application of formic acid on wilted grass was examined but the analytical results of the silages from the test-tube silos confirm that formic acid does preserve the WSC. The value of high WSC contents in silage to the animal and the rate of deterioration of silages of this type after removal from the silo must be investigated before the use of formic acid on wilted crops can be generally recommended.

VII. THE EFFECT OF SUNLIGHT ON THE WATER SOLUBLE CARBOHYDRATE CONTENT OF RYEGRASSES.

VII. THE EFFECT OF SUNLIGHT ON THE WATER SOLUBLE CARBOHYDRATE CONTENT OF RYEGRASSES.

VII. (I). INTRODUCTION.

The experiments reported in this section terminated a series in which the WSC contents of grasses and clovers were studied. The results of the earlier work, in which the effect on the WSC content of species, variety, stage of growth, nitrogenous fertiliser and time of cutting were considered, were reported in earlier publications (McDonald and Henderson, 1961, 1962, 1963, 1964b, 1965, 1966). The aim of the experiments reported here was to discover the extent to which quotidian variation in sunlight affects the WSC contents of ryegrasses.

The effect of light intensity on WSC content under controlled environmental conditions has been established by several workers (Alberda, 1957, 1965b; Bathurst and Mitchell, 1958; Deinum, 1966; Deinum and Dirven,1972). In the field, Waite and Boyd (1953a) noted that relatively small climatic differences caused considerable variation in the rate and type of growth, and in the pattern of WSC content over the growth season. Witt (1970) claimed that, apart from the first week after cutting, the climatic conditions may have a more important influence on the concentration of WSC in grassland plants than the stage of growth. Under bad weather conditions, he found the WSC contents of ryegrasses to be almost as low as those of legumes.

VII. (2) EXPERIMENTAL AND RESULTS.

The grass used in the first experiment (G1) was the first cut of the season of an established sward of Lolium perenne at a leafy stage of growth. The plot ($84.3m^2$) was divided into 384 equal areas, 16 of which were cut at random each day over the period 11 May to 3 June and the harvested grass was bulked and thoroughly mixed in order to obtain a representative sample. The grass was cut at 10.00h each day to minimise the error due to diurnal variation. The CF

contents ranged from 17.1 per cent of DM (day 1) to 19.8 per cent of DM (day 24) and the cellulose contents ranged from 20.8 per cent of DM (day 1) to 22.6 per cent of DM (day 24). The daily samples were analysed for DM, WSC and CP contents and the results, along with hours of sunshine and ground and air temperatures, are given in Table 46. Hours of sunshine and temperatures (taken at 10.00h) were recorded at a Meteorological Sub-station situated a short distance from the plots. The percentage WSC in DM and the hours of sunshine during the previous 34h period are shown in Figure 18.

TABLE 46.

Chemical composition of Lolium perenne, hours of sunshine and temperatures over the 24 day period.

Date	Day No.	%DM	% WSC in DM	% CP in DM	Hours sunshine	Air temp. at ground level (°C)	Ground temperature (°C)
11 May	1	19.4	13.91	25.09	0	10.2	8.6
12 "	2	16.9	11.51	23.66	5.9	9.7	8.8
13 "	3	19.0	16.46	22.76	4.8	11.0	8.2
14 "	4	19.4	17.40	21.88	7.9	12.0	9.2
15 "	5	19.3	16.97	21.00	13.1	10.0	8.6
16 "	6	20.8	20.66	21.54	9.8	16.5	10.3
17 "	7	17.0	19.06	19.82	3.2	10.0	10.9
18 "	8	20.6	19.47	19.30	6.4	15.8	8.8
19 "	9	18.0	17.28	19.73	7.0	10.0	9.1
20 "	10	17.5	18.58	18.56	8.8	9.5	8.3
21 "	11	16.2	18.99	19.29	1.7	9.5	9.3
22 "	12	16.6	17.78	18.17	1.9	11.9	11.4
23 "	13	18.9	18.78	17.77	10.1	8.8	9.0

TABLE 46. (contd.)

Date	Day No.	%DM	% WSC in DM	%CP in DM	Hours sunshine	Air temp. at ground level (°C)	Ground temperature (°C)
24 May	14	20.8	21.87	16.26	1.3	10.5	8.8
25 "	15	18.2	19.29	17.75	0.9	11.0	10.6
26 "	16	19.0	21.19	16.47	3.5	16.8	10.1
27 "	17	19.1	19.84	16.16	5.5	9.2	9.8
28 "	18	20.9	24.70	15.03	11.7	13.0	9.9
29 "	19	21.5	26.49	15.19	12.1	17.8	12.1
30 "	20	20.8	24.81	15.02	13.7	18.8	13.1
31 "	21	20.0	24.44	15.79	11.0	18.7	14.5
1 June	22	21.2	26.50	14.85	6.8	15.5	14.9
2 "	23	21.4	26.47	13.71	2.9	13.0	14.2
3 "	24	21.1	24.50	13.68	3.5	13.3	13.3

The experiment was repeated using an autumn (third) cut of Lolium multiflorum sown in the spring (G 2). The plot had been cut previously on 6 July and 17 August and had received 50 kg N/ha after each cut. The grass, harvested during the period 30 August to 22 September, was at a leafy stage of growth and sampling and analytical techniques were the same as those used in the previous experiment. The CF contents of the grass ranged from 19.8 per cent of DM (day 1) to 18.9 per cent of DM (day 24) and the cellulose contents ranged from 24.4 per cent of DM (day 1) to 23.0 per cent of DM (day 24). The results of chemical analysis, hours of sunshine and air and ground temperatures are given in Table 47 and the percentage WSC in DM and hours of sunshine during the previous 34 h are shown in Figure 19.

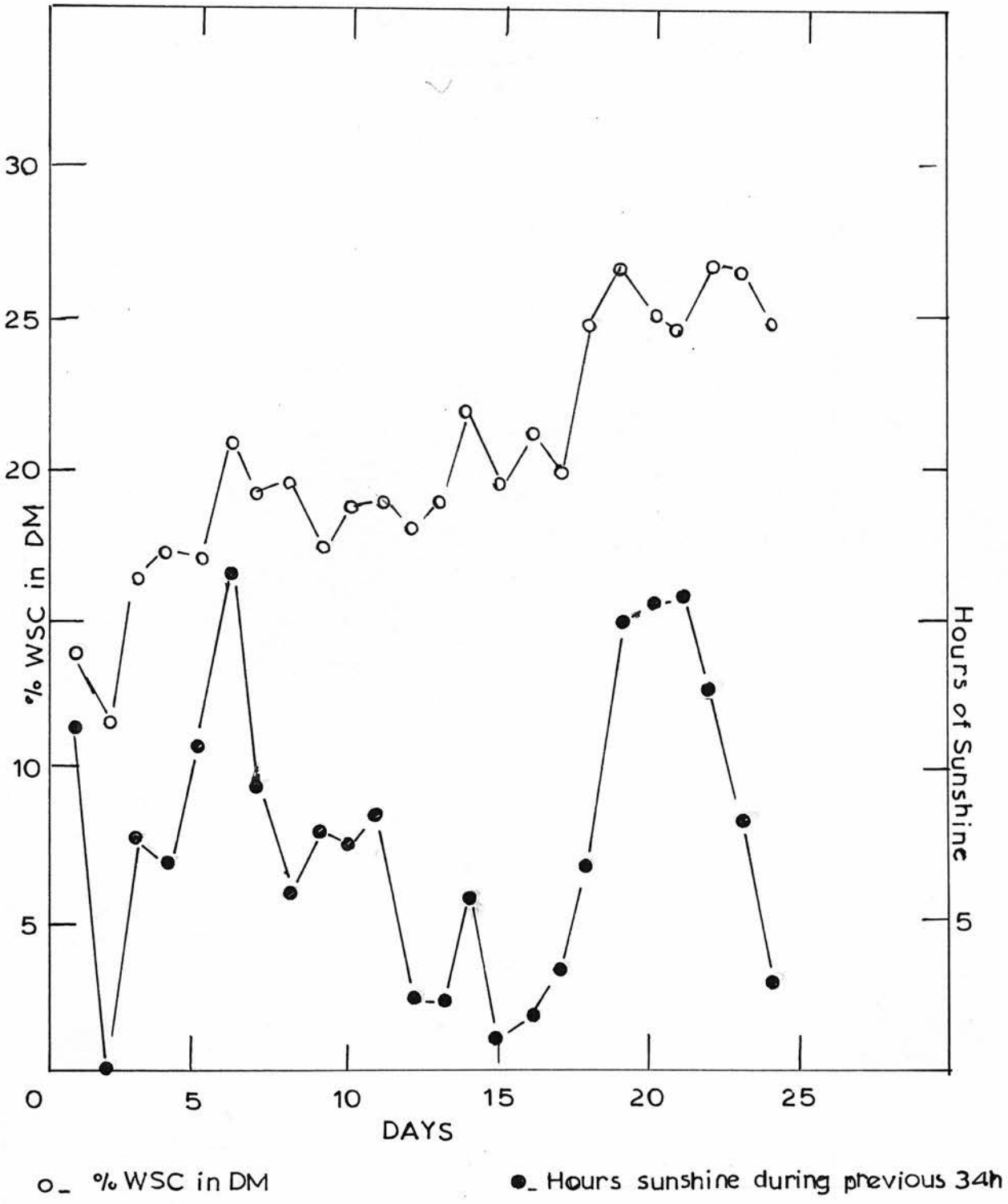


Fig. 18. Relationship between WSC content and sunshine EXPERIMENT G1.

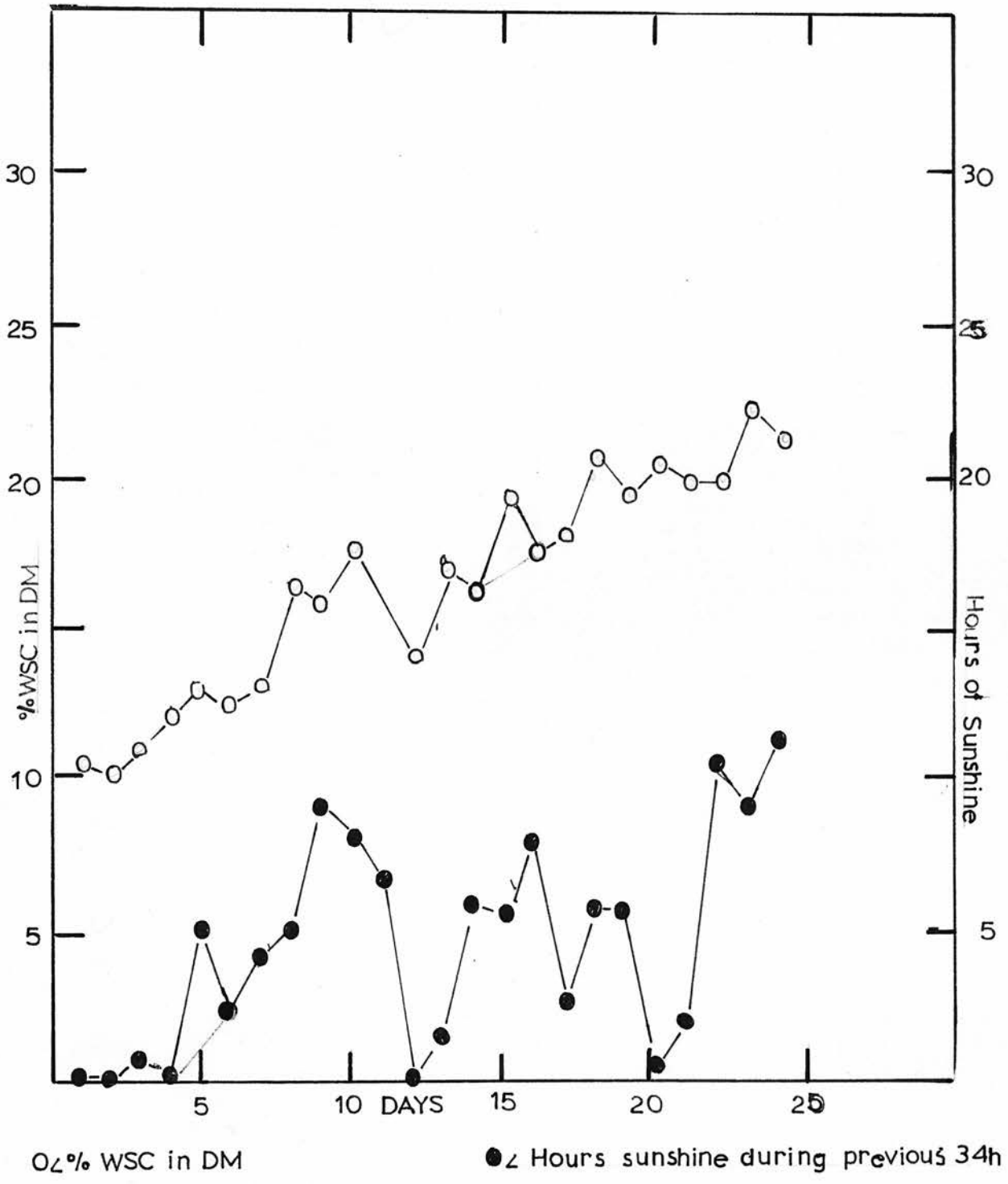


Fig. 19. Relationship between WSC content and sunshine

EXPERIMENT G2.

TABLE 47.

Chemical composition of Lolium multiflorum, hours of sunshine and temperatures over the 24 day period.

Date	Day No.	%DM	%WSC in DM	%CP in DM	Hours sunshine	Air temp. at ground level (°C)	Ground temperature (°C)
30 August	1	11.4	10.47	21.3	0	12.5	13.2
31 "	2	11.7	10.27	21.2	0.7	12.5	12.7
1 September	3	12.2	10.98	21.1	0.2	14.9	12.3
2 "	4	11.9	12.21	19.4	5.0	14.8	12.8
3 "	5	15.1	12.93	19.0	2.3	14.7	12.7
4 "	6	11.1	12.43	18.9	4.0	12.8	13.2
5 "	7	15.1	12.89	19.0	3.0	13.2	11.7
6 "	8	17.7	16.34	16.3	9.0	15.1	12.5
7 "	9	17.8	15.63	16.6	6.3	14.7	11.2
8 "	10	18.4	17.48	15.5	6.8	13.2	10.8
9 "	11	16.6	15.91	16.3	0	11.8	11.1
10 "	12	12.8	13.87	17.1	0.1	14.2	12.3
11 "	13	14.1	16.91	17.0	5.8	14.3	12.1
12 "	14	12.6	16.11	16.1	4.2	13.6	13.4
13 "	15	14.5	19.18	15.4	8.0	14.0	11.6
14 "	16	12.6	17.35	15.8	2.1	12.2	11.4
15 "	17	13.7	17.88	15.2	5.0	9.5	10.6
16 "	18	18.9	20.52	14.5	5.6	10.6	9.2
17 "	19	14.3	19.26	14.6	0.5	13.1	11.0
18 "	20	15.0	20.36	14.4	0	13.4	12.4
19 "	21	16.5	19.69	13.6	9.6	16.0	11.6

TABLE 47. (contd.)

Date	Day No.	%DM	%WSC in DM	%CP in DM	Hours sunshine	Air temp.at ground level (°C)	Ground temperature (°C)
20 September	22	19.6	19.82	13.4	7.3	15.1	12.6
21 "	23	19.7	22.12	13.0	9.6	13.4	10.9
22 "	24	15.4	21.06	12.8	1.8	13.1	10.3

VII. (3) DISCUSSION.

There was an obvious stage of growth effect on the CP contents of the grasses in both experiments, the levels decreasing as the grass matured. In experiment G1, the regression of CP percentage with day number (w) is % CP = 23.79 - 0.44 w and the correlation coefficient (r) is -0.974. In experiment G2, the regression of CP percentage with day number (w) is % CP = 20.59-0.35 w and the correlation coefficient (r) is -0.955. In experiment G1, the grass had not headed by the 24th day and in experiment G2, a few heads were visible on the 19th day. As would be expected, in experiment G1, the CF and cellulose contents of the grass cut on the 24th day were higher than the CF and cellulose contents of the grass cut on the first day. The CF and cellulose contents of the grass cut on the 24th day in experiment G2 are difficult to explain.

Alberda (1957) claimed that the WSC contents of grasses are high in winter and that, during the spring and early summer when growth is rapid, they fall to much lower values and rise again during August and September. These findings do not agree with those of Waite and Boyd (1953a) and others who found a rise in WSC content in spring and a fall in autumn. Under grazing conditions, however, Waite and Boyd (1953b) obtained a different pattern of results, with high values in May and June, low in July and August, and high in September.

In a field trial, Spiertz and Ellen (1972) found that the total WSC content of tillers and stubble increased exponentially up to 14 days after flowering, after which it remained at a constant level or decreased. In the experiments reported here, there was a positive correlation between WSC content and stage of growth. There was considerable deviation from the regression line, however, and an attempt was made to correlate this with hours of sunshine during the 34 hours prior to cutting. The regression of percentage WSC in DM on hours of sunshine was calculated for the first experiment and yielded the fitted regression relationship $y = 11.94 + 0.27x + 0.49w$ where y = % WSC, x = hours of sunshine and w = day number. A test for autocorrelation was performed and the Durbin - Watson 'd' (Durbin and Watson, 1950, 1951) was found to be 1.79 which is not significant at the 2.5 per cent level. The partial correlation coefficient $r_{xyw} = 0.744$ may be considered an adequate representation of the relationship, hours of sunshine (x) and percentage of WSC in DM (y). In the second experiment, the value for 'd'was found to be 2.4 which is not significant at the 5% level and the partial correlation coefficient was found to be $r_{xyw} = 0.343$. This bears out the results of the first experiment, although the relationship is somewhat weaker. A similar analysis of the results of the first experiment, relating the effect of the hours of sunshine on CP per cent in the DM of the grass was considered but there was no significant relationship. In the second experiment, there was a significant negative relationship between these variables ($r_{zxw} = -0.52$) where z = % CP in DM x = hours sunshine and w = day number. Deinum (1966) found that while DM production, DM percentage and WSC content of Lolium perenne increased with higher light intensity, CP, CF, ash and nitrate-N decreased.

In conclusion, these experiments demonstrate that the WSC contents of rye-grasses grown in the east of Scotland, and at a leafy stage of growth may be influenced by the sunlight during the 34 hours prior to cutting. This is consistent with the findings of Wylam (1957) who correlated the sucrose content

of the whole plant with sunshine and found that the sucrose peaks coincided with maximum hours of sunshine on the day before cutting. Van der Schaaf (1967), working in Holland, also related the WSC content of first cut grass to hours of sunshine on the sampling day and the day before, and to the temperature during the growing period and obtained the following regression equation:-

$$S = 0.39Z - 0.80T + 19.1$$

where S = % WSC, Z = hours sunshine and T = average daily temperature.

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X. APPENDIX 1 - METHODS OF ANALYSIS.

X (I) Determination of dry matter in silage and silage effluent by Toluene Distillation.

Samples of 65g of chopped silage or 60ml of effluent are accurately weighed into a distillation flask and heated with about 400 ml toluene in a specially constructed distillation apparatus. The distillation is continued for 7-8h at a rate of 2-3 drops per second. At the end of this period, a fine jet of toluene is directed down the condenser to remove the last traces of water and the distillation continued for a further 15 minutes.

Before the volume of water is read, the graduated trap is immersed in a water bath at 20°C for 20 minutes. The apparatus is disconnected and the aqueous layer transferred into a 100 ml graduated flask and made up to the mark with CO_2 - free distilled water.

The total acid content of each distillate is determined on an aliquot of the diluted distillate by the method of Foreman (1920). Samples of 20 ml of the diluted distillates are diluted with 80 ml of neutral ethanol and titrated with 0.1N - NaOH to the phenolphthalein end point.

The calculation of DM is as follows

$$D = 100 - 99.8\ (V - 0.0055T)/W$$

where D = % dry matter, V = observed volume of distillate.

T = total titre of distillate (ml of 0.1N - NaOH)

W = weight of sample in g.

X (2) Determination of the buffering capacity of grass or silage.

Ten g samples of chopped grass or silage are macerated with 250ml chilled distilled water for two minutes. The macerate is transferred to a beaker, stirred constantly with a magnetic stirrer and its pH value is recorded.

The above macerate is titrated to pH 3 with 0.1N HCl, and then titrated to pH 6 with 0.1N NaOH. The volume of standard alkali required

between pH 4 and pH 6 is recorded. The macerate is acidified first to remove bicarbonate which would act as a buffer if present.

The buffering capacity is expressed as - mg equiv. of alkali required to change the pH from 4-6 per 100 g dry matter.

X (3) Nitrogen distribution in grass and silage.

Nitrogen distribution should always be determined in fresh undried material with the possible exception of total nitrogen analyses on fresh grass, where drying will not normally result in significant loss of N. Otherwise drying will result in distributional changes in grass and loss of volatile N from silage.

TOTAL N (TN) is determined by the usual Kjeldahl methods. If it is desired to include nitrate-N a reduction procedure using Devarda alloy or similar reagent should precede digestion.

TOTAL SOLUBLE N or NON PROTEIN N (NPN) which gives a measure of the degree of proteolysis is found by extracting a sample twice with boiling water, making to volume and determining TN of the filtrate by micro-Kjeldhal. Generally a 100g sample is extracted to a final volume of about 1500 ml and no concentration of the filtrate is necessary.

VOLATILE N (VN) which measures the extent of deamination and is a guide to the quality of silage, is determined on the NPN filtrate by steam distillation in the micro-Kjeldahl apparatus using excess M/20 sodium borate (pH ca. 9.2) to liberate ammonia. A 5-10 ml sample of filtrate should be sufficient.

X (4) Determination of nitrate - N in dried grass and silage.

One g of air-dried ground material is extracted with 100 ml of cold distilled water, shaken for 5 minutes using a shaking machine, then

filtered through Whatman No. 54 paper and washed. Duplicate extractions are made on each sample. To one extract is added I g of magnesia and to the other I g of magnesia and 3 g of Devarda's alloy. Both extracts are distilled for half an hour into .025N sulphuric acid, the difference between the two determinations giving the nitrate-N.

X (5) Determination of water soluble carbohydrates in grass or silage.

Twenty-five g samples of chopped grass or silage are macerated with 200 ml of chilled, distilled water in a macerator for three minutes. The fibrous residue is removed by filtration through cloth and I00 ml of the filtrate are transferred to a conical flask, acidified with 0.IN - sulphuric acid to pH 4 and brought to the boil to coagulate proteins. Celite is added to aid filtration. The proteins are removed by filtration through Whatman No. I paper and the residue is washed with distilled water. The filtrate is made up to 250 ml. Hydrolysis is effected by adding five ml of 2N-sulphuric acid to I5 ml of the diluted filtrate in a boiling tube fitted with a cold-finger condenser and immersing the tube in a boiling water bath for ten minutes. After being cooled for five minutes the hydrolysate is made neutral to methyl red with sodium hydroxide and made up to volume (50-I00 ml). Aliquots of five ml are analysed for reducing sugars by the modified Somogyi reagent of Wiseman et al (I960).

In the preparation of a grass or silage extract for the determination of individual water soluble carbohydrates the I00 ml aliquot is neutralised with 0.IN-sodium hydroxide before coagulation of the protein by boiling. The filtrate is passed through a mixed resin column (20cm x I.3cm diameter) containing equal amounts by wet volume of IRC-50(H) and IR-45(OH). The resin is washed with 200 ml water and the combined filtrate and washings are reduced in volume (I0-50 ml) in a rotary

evaporator at 40° - 50° C.

Two spots of the extract are applied I cm from the edge of a sheet of 3MM chromatography paper. An accurately known volume of the extract (0.5ml) is streaked between these two spots, keeping the streak as narrow as possible. Eight to ten applications are required. Between each application it is allowed to dry completely. The end of the strip furthest from the extract streak is cut to form a serrated edge from which solvent can drip in a uniform manner. The chromatogram is placed in a tank presaturated with the eluting solvent, ethyl acetate; acetic acid; formic acid; water (I8; 3; I; 4), and left for 32 hours. The paper is removed and allowed to dry completely in a fume cupboard.

The 2.5 cm marginal strips are removed and dipped into silver nitrate reagent prepared by saturating two ml of water with silver nitrate, adding 400 ml of acetone and water dropwise until the precipitate which forms disappears. The strips are allowed to dry (5-I0 minutes) and are then sprayed with alcoholic sodium hydroxide (I vol alcohol + I vol 5N-sodium hydroxide). When the brown-black spots are clearly visible (I-I0 minutes depending on the sugars present) the brown background colour is removed by dipping the chromatogram through I0 per cent sodium thiosulphate solution, washing it in running water and allowing it to dry. The sugars are identified by running standards and their positions are marked on the chromatogram which is then cut into strips each containing one sugar. Small pieces of Whatman No. I filter paper are stapled to each end of the strips and the sugars are eluted in an airtight container with 5-I0 ml of water. The sugar present is then determined by a modified version of the Somogyi method using the appropriate sugar (0.25 mg-I.0mg / 5 ml)

as a standard. Fructans are determined by the Roe method (Arni and Percival, 1951) and oligosaccharides, including sucrose, after a four hour hydrolysis in 0.5N-sulphuric acid. A correction is made to this figure on the assumption that 31.7 per cent of the fructose is destroyed during this hydrolysis.

X (6) Determination of mannitol in silage.

Mannitol is separated from the sugars on a chromatogram, as above, and eluted from the paper. One ml of sodium periodate (0.25M) is added to five ml of mannitol solution (0.2-3.0mg) in a boiling tube and the tube is stoppered and heated in a boiling water bath for 20 minutes to convert the mannitol to formic acid. The tube is cooled under running water for five minutes; four drops of ethylene glycol are added with shaking. After five minutes a spatula of potassium iodide and 15 ml of 0.01N -sodium thiosulphate are added to the boiling tube and the contents are shaken and left in the dark for ten minutes. The iodine produced reacts with an equivalent amount of thiosulphate and the remaining thiosulphate is determined by titration with standard iodine solution (0.01N). The recovery of formic acid from a known weight of mannitol is determined using standard mannitol solutions.

X (7) Determination of ethanol in silages and silage effluents.

Twenty-five grams of chopped silage are macerated with 200 ml of chilled, distilled water in a macerator for three minutes. The fibrous residue is removed by filtration through cloth and 20 ml of this filtrate or 20 ml of effluent (diluted if necessary) are measured into a distillation tube and the protein is precipitated by the addition of five ml of 10 per cent sodium tungstate and five ml of 1N-sulphuric acid. Ten ml of a saturated solution of mercuric chloride and 10 ml of a 10N solution of

sodium hydroxide are measured into a second tube. The tubes are then connected to the distillation apparatus and immersed in a boiling water bath which should be kept at the boiling temperature during the distillation process. The alcohol is steam-distilled directly into a digestion flask. After 25 to 30 ml are distilled, I0 - 25ml of .04N-potassium dichromate solution and 5ml of concentrated sulphuric acid are added to the distillate. The sulphuric acid should be allowed to run down the side of the flask to prevent it from mixing with the aqueous solution and causing the heat of solution to raise the temperature before the flask is closed. After the flask is closed and the cap fastened with two springs, the solution is mixed. The flask is then placed in a boiling water bath for 20 minutes. The solution is cooled, and the dichromate solution is washed down the sides of the flask with a stream of water from a wash bottle. The solution is transferred to a 500 ml beaker, the flask is washed and the solution is diluted to 400 ml with recently boiled and cooled distilled water. Two g of potassium iodide are added and the iodine liberated is titrated with 0.02N-sodium thiosulphate using a starch indicator. Each ml of 0.IN-sodium thiosulphate reduced is equivalent to I.I5 mg ethanol. All glassware should be cleaned with hot chromic acid and a trace of distilled water should be used to lubricate joints.

X (8) Determination of organic acids in grass or silage.

Preparation of acid extract.

Fifty g of finely chopped fresh material are placed in a 4oz storage bottle. Fifty ml (or more if the DM of the herbage is high) of 0.6N-sulphuric acid are added and the sample is carefully tamped below the level of the

acid. A crystal of thymol is added and the bottle is sealed and stored at 2-5°C for one week. During this period the sample must remain covered with acid.

After one week the contents of the bottle are throroughly mixed and squeezed through muslin and the filtrate centrifuged to remove solid particles. The extract is then stored in the deep freeze.

Preparation of solvents.

I. Benzene - 200 ml of 0.5N-sulphuric acid are added to I600ml of pure benzene in a large separating funnel and the contents are shaken for two minutes. The two phases are allowed to separate and the aqueous layer is discarded. The remaining water droplets are removed from the benzene by filtering it through a fluted I8.5 cm phase separating paper. The solvent is stored in a dry, sealed storage bottle.

2. 80 per cent benzene/butanol. 200ml of 0.5N-sulphuric acid are added to I600 ml pure benzene and 400 ml butanol in a large separating funnel and the contents are shaken for two minutes. The two phases are separated and the solvent filtered as above.

Preparation of silicic acid.

Two hundred g of silicic acid are added to I800ml of 2N-hydrochloric acid in a large beaker. The suspension is stirred until it is homogeneous and then heated on a hot plate to near boiling point (ca 90°C) for I-2h with constant stirring. The suspension is allowed to cool for I-2h with occasional stirring, the gel is allowed to settle and its level is marked. The hydrochloric acid and fines are decanted off and the beaker is filled up with distilled water, the gel is allowed to settle and the liquid is again decanted off. The washing with water is repeated 8-I0 times until the washings are free from chloride and the gel has been reduced by half.

After the last washing the gel is transferred to a large Buchner funnel and sucked dry. Ethanol is poured through the gel and it is again sucked dry. The gel is transferred to a shallow tray and dried at 105°C-110°C overnight. The gel is now a free-running powder and is stored out of contact with the air.

Preparation of column.

Ten g of silicic acid are placed in a 50ml beaker and 6ml of 0.5N-sulphuric acid are added. The mixture is stirred until it once again becomes a free-running powder. The gel is then slurried with 25ml of benzene solvent.

The bottom of a glass column (20cm useful length x 1.3cm internal diameter) is packed with a pad of glass wool which has been washed in a weak acid solution, thoroughly washed with distilled water and dried. The slurry is added to the column and a tamping rod is passed up and down the column twice to remove air bubbles. Gentle pressure is applied with the tamping rod until the surface of the gel is firm. All gel is removed from the sides of the column and a pad of thick filter paper is placed on the surface of the silicic acid. The pad is pressed firmly on to the surface and the solvent is allowed to pass through the column until it is within 0.5cm of the pad.

Application of acids to the column.

The sample (0.4ml of the extract) is applied to the filter pad dropwise followed by 0.1ml of 10N-sulphuric acid. The acid sample is washed into the pad with the solvent remaining in the column and then twice more with 2-4ml aliquots of benzene solvent. Twenty-five ml of benzene solvent are added to the column and fractions (approx 3.5ml) are collected in a fraction collector and titrated with 0.005N-sodium

hydroxide using thymol blue (0.5ml per tube) as indicator.

A mixing chamber with side arm, containing 350 ml benzene solvent is fitted to this column. The reservoir, containing 400 ml of benzene/ butanol solvent is fitted above the mixing chamber and when the benzene solvent in the column is within 0.5cm of the filter pad the gradient elution is allowed to commence. The fractionation is allowed to continue until lactic acid has been removed from the column.

A standard solution of the acids present in the samples is applied to a similar column and the percentage recovery of the acids is calculated.

X (9) Determination of modified acid detergent fibre in grass or silage.

One g of finely ground (0.6 mm mesh) dried grass or silage is weighed into a 500 ml conical flask. One hundred ml of modified acid detergent (I% W/V of cetyl trimethyl ammonium bromide in IN-sulphuric acid) are added and the contents of the flask are brought to the boil and allowed to boil gently under reflux for 2h. The residue is filtered off on a dried, weighed sintered glass crucible (porosity - I; capacity - 50ml). The flask is washed three times with boiling water and the residue five times with boiling water (approx I00ml of water in all). The residue is washed once with alcohol and dried overnight (I6h). The crucible is cooled in a desiccator and weighed and the weight of MAD-F in one g of dried herbage is found by difference.

X (I0) Determination of cellulose in grass or silage.

Fifteen ml of 80 per cent acetic acid and I.5 ml concentrated nitric acid are added to Ig dried grass or silage in a I50 ml round-bottomed, wide-necked flask fitted with a reflux condenser. The contents of the flask are boiled gently for 20 minutes and then cooled. Twenty ml of ethanol are added and the contents of the flask are filtered through a

a sintered glass crucible (porosity I; capacity 50 ml) with suction. The residue is washed with ethanol until free from acid and then with benzene. The crucible and contents are dried at 100°C overnight and weighed, ignited at 600°C and reweighed. The loss on ignition represents cellulose.

XI. APPENDIX 2 - RESULTS.

XI (1) EXPERIMENT 01 - TABLE A1.

Percentage DM

Treatment		A		B		C		D	
Silo		1	2	3	4	5	6	7	8
Sample									
Top	1	18.9	21.9	19.6	15.5	18.9	20.3	18.9	20.5
	2	19.8	21.8	18.0	16.0	20.1	22.9	16.5	24.1
	3	19.2	21.1	15.9	17.5	19.6	23.7	14.3	22.0
Middle	1	18.6	19.7	21.3	19.6	19.4	18.8	20.4	21.0
	2	19.8	20.0	20.7	20.2	19.7	19.8	19.6	19.5
	3	19.5	23.0	20.5	20.0	19.6	21.2	19.9	19.4
Bottom	1	19.5	18.3	20.1	17.8	19.4	20.6	20.0	20.5
	2	18.8	21.0	21.2	19.7	20.4	19.9	19.8	20.2
	3	19.1	19.8	21.3	20.0	20.8	19.3	20.1	19.5

XI (2) EXPERIMENT 01 - TABLE A 2.

pH

Treatment		A		B		C		D	
Silo		1	2	3	4	5	6	7	8
Sample									
Top	1	4.35	4.57	4.05	4.72	5.87	5.74	6.19	6.29
	2	4.24	4.37	4.24	4.40	5.72	5.80	6.63	6.46
	3	4.42	4.49	4.32	4.56	5.82	5.99	6.54	6.20
Middle	1	4.23	4.39	4.80	4.79	5.79	5.64	5.01	5.53
	2	4.22	4.15	4.75	4.45	5.38	5.20	4.31	4.72

EXPERIMENT 01 - TABLE A2. (contd.)

Treatment		A		B		C		D	
Silo		1	2	3	4	5	6	7	8
Middle	3	4.34	4.19	4.39	4.12	5.70	5.27	4.69	4.71
Bottom	1	4.30	4.23	4.20	4.00	5.82	5.74	4.57	4.05
	2	4.28	4.25	4.03	4.07	5.95	5.55	4.90	4.31
	3	4.43	4.15	4.05	4.06	5.63	5.95	4.75	4.36

XI (3) EXPERIMENT 01 - TABLE A3.

Percentage WSC in DM

Treatment		A		B		C		D	
Silo		1	2	3	4	5	6	7	8
Sample									
Top	1	4.0	3.4	2.0	1.1	19.9	21.4	22.6	18.6
	2	2.8	2.0	1.3	0.5	17.9	17.5	12.0	17.1
	3	5.0	2.6	1.4	1.7	16.0	14.3	6.1	27.4
Middle	1	5.3	7.8	3.4	4.1	24.7	19.1	20.5	18.5
	2	9.8	10.6	15.5	20.0	23.1	21.0	15.5	24.5
	3	7.7	8.5	15.8	13.9	23.3	21.6	16.7	25.1
Bottom	1	15.9	5.8	4.6	3.8	23.5	27.6	16.9	12.8
	2	12.6	17.8	10.0	19.1	27.2	24.9	21.1	16.3
	3	11.7	17.1	9.8	13.8	28.5	26.5	22.4	15.6

XI (4) EXPERIMENT 01 - TABLE A4

Percentage MAD-F in DM.

Treatment		A			B		C		D
Silo		1	2	3	4	5	6	7	8
Sample									
Top	1	32.9	34.3	34.7	41.7	27.9	29.9	33.5	30.3
	2	28.6	34.7	38.7	43.5	27.7	31.4	37.1	29.9
	3	30.1	31.8	40.7	44.0	27.4	30.4	39.1	27.8
Middle	1	28.8	36.6	34.4	37.1	27.3	29.0	30.1	29.7
	2	29.2	28.8	31.2	29.0	28.9	28.4	28.1	32.0
	3	29.5	27.7	29.9	30.1	27.9	29.0	28.7	31.5
Bottom	1	28.2	28.1	29.8	29.5	29.4	27.4	29.8	29.8
	2	28.0	28.3	29.3	29.4	29.9	28.0	28.8	31.1
	3	29.9	26.6	29.9	32.2	28.1	26.3	27.9	29.4

XI (5) EXPERIMENT 01 - TABLE A5

Percentage organic acids in DM.

Treatment	Silo	Sample		Acetic	Propionic	Butyric	Lactic	Succinic
A	1	Top	2	0.9	0.6	2.3	6.7	1.8
		Bottom	2	0.9	0.1	0.2	7.1	0.7
	2	Top	2	1.1	0.2	3.2	4.1	1.7
		Bottom	2	0.8	0.1	0.6	8.6	1.8
B	3	Top	2	3.0	0.2	1.4	2.9	0.2
		Bottom	2	0.7	0.1	0.3	3.5	0.2
	4	Top	2	2.8	0.2	2.1	1.8	0.1
		Bottom	2	1.4	0.1	0.4	6.9	0.8

EXPERIMENT 01 - TABLE A5 (contd.)

Treatment	Silo	Sample		Acetic	Propionic	Butyric	Lactic	Succinic
C	5	Top	2	0.2	0.1	0.6	0.2	0.7
		Bottom	2	0.3	0.1	0.1	0.1	0.6
	6	Top	2	0.2	0.1	0.2	0.4	1.2
		Bottom	2	0.2	0.1	0.2	0.6	0.7
D	7	Top	2	0.3	0.3	0.3	0.1	0.2
		Bottom	2	0.8	0.2	0.2	3.3	0.7
	8	Top	2	0.3	0.2	0.2	0.1	0.1
		Bottom	2	1.0	0.2	0.5	4.8	0.4

XI (6) EXPERIMENT 01 - TABLE A6

Microbial assay of individual silage samples.
(counts/10g silage)

Treatment	Silo	Sample		VRBA	TAA	MEA	YEA	LF	PC
A	1	Top	1	$<10^2$	1.5×10^7	2.0×10^2	3.1×10^8	10^2	10^3
			2	"	3.4×10^7	10^2	3.9×10^8	10	10
			3	"	1.7×10^7	10^2	4.0×10^7	10^2	10^3
		Middle	1	")	3.7×10^7	10^3	2.1×10^7	10	10^2
			2	")					
			3	"	1.0×10^7	10^2	1.1×10^7	10	10^2
		Bottom	1	")	8.2×10^7	2.7×10^2	1.4×10^8	10	10
			2	")					
			3	"	8.8×10^7	5.1×10^2	9.0×10^7	10	10^2
	2	Top	1	"	1.6×10^6	5.0×10	10^7	10	10^2
			2	"	1.5×10^7	10^2	1.8×10^7	10^2	10^3
			3	"	1.0×10^8	3.0×10^2	1.1×10^8	10	10^2

EXPERIMENT 01 - TABLE A6 (contd.)

Treatment	Silo	Sample		VRBA	TAA	MEA	YEA	LF	PC
A	2	Middle	1	$<10^2$	5.7×10^7	10^2	5.1×10^7	10	10
			2	"	1.7×10^8	5.0×10	1.4×10^8	<10	10
			3	"	4.1×10^7	10^2	3.7×10^7	10	10
		Bottom	1	"	3.7×10^7	4.0×10^3	3.0×10^7	10	10^2
			2	"					
			3	"	2.4×10^8	2.1×10^4	2.1×10^8	10	10
B	3	Top	1	"	1.8×10^8	3.7×10^4	1.1×10^8	<10	10
			2	"	1.9×10^7	10^2	2.6×10^7	10	10^2
			3	"	5.5×10^8	10^2	7.1×10^8	10	10^2
		Middle	1	"	4.9×10^8	5.0×10	5.5×10^8	10^3	10^2
			2	"	3.2×10^7	5.0×10	4.3×10^8	10^3	10^2
			3	"	1.9×10^7	10^2	2.3×10^7	10^2	10^3
		Bottom	1	"	2.8×10^8	10^2	3.4×10^8	10	10^3
			2	"	2.6×10^8	1.1×10^3	2.2×10^8	10^2	10^2
			3	"					
	4	Top	1	"	3.2×10^7	5.0×10	7.1×10^7	10^3	10^2
			2	"	4.9×10^7	10^2	5.6×10^7	10	10^3
			3	"	3.5×10^7	10^2	9.4×10^7	10	10^2
		Middle	1	"	3.4×10^7	$<5.0\times10$	3.1×10^8	10^3	10^2
			2	"	2.6×10^8	10^2	3.4×10^8	<10	10^3
			3	"	2.3×10^8	2.0×10^3	2.3×10^8	<10	10
		Bottom	1	"	2.1×10^8	10^3	1.8×10^8	<10	10
			2	"	5.3×10^8	3.0×10^3	4.9×10^8	10	10^2
			3	"					

EXPERIMENT 01 - TABLE A6 (contd.)

Treatment	Silo			VRBA	TAA	MEA	YEA	LF	PC
C	5	Top	1	-	6.9×10^{8}	1.2×10^{6}	4.9×10^{9}	$<10^{3}$	$<10^{2}$
			2	-	3.3×10^{8}	3.8×10^{6}	1.6×10^{10}	$<10^{6}$	$<10^{2}$
			3	-	5.6×10^{8}	-	4.8×10^{9}	-	-
		Middle	1	-	4.0×10^{8}	1.1×10^{7}	1.9×10^{9}	-	-
			2	-	5.5×10^{8}	7.7×10^{6}	1.6×10^{9}	-	-
			3	-	2.3×10^{8}	3.7×10^{6}	3.1×10^{8}	-	-
		Bottom	1	-	6.3×10^{7}	-	1.7×10^{9}	-	-
			2	-	6.4×10^{7}	9.4×10^{6}	1.0×10^{9}	-	-
			3	-	4.0×10^{8}	9.5×10^{6}	5.0×10^{9}	-	-
	6	Top	1	-	3.4×10^{8}	1.3×10^{6}	1.3×10^{10}	$<10^{3}$	$<10^{3}$
			2	-	6.3×10^{8}	1.3×10^{6}	1.6×10^{10}	$<10^{3}$	<10
			3	-	1.9×10^{8}	6.4×10^{4}	1.1×10^{10}	$<10^{2}$	$<10^{3}$
		Middle	1	-	6.5×10^{8}	1.2×10^{4}	7.7×10^{9}	<10	$<10^{2}$
			2	-	4.0×10^{8}	1.3×10^{4}	3.9×10^{9}	<10	$<10^{3}$
			3	-	5.8×10^{8}	8.0×10^{6}	1.5×10^{9}	$>10^{6}$	<10
		Bottom	1	-	4.2×10^{8}	2.0×10^{7}	1.8×10^{9}	<10	<10
			2	-	7.5×10^{8}	-	6.1×10^{11}	<10	<10
			3	-	3.9×10^{8}	1.1×10^{7}	2.7×10^{9}	$<10^{3}$	-
D	7	Top	1	-	1.6×10^{5}	1.9×10^{5}	1.6×10^{10}	<10	<10
			2	-	2.4×10^{5}	1.4×10^{4}	4.5×10^{9}	$<10^{3}$	<10
			3	-	5.7×10^{5}	1.8×10^{5}	2.5×10^{10}	<10	<10
		Middle	1	-	2.0×10^{7}	6.0×10^{6}	5.0×10^{7}	<10	$<10^{2}$
			2	-	1.1×10^{7}	4.3×10^{6}	3.0×10^{7}	$<10^{4}$	$<10^{3}$
			3	-	4.0×10^{8}	1.6×10^{7}	1.7×10^{9}	$<10^{4}$	$<10^{3}$
		Bottom	1	-	8.1×10^{8}	$>2.0\times10^{8}$	1.9×10^{9}	-	$<10^{2}$

EXPERIMENT 01 - TABLE A6 (contd.)

Treatment	Silo	Sample		VRBA	TAA	MEA	YEA	LF	PC
D	7	Bottom	2	-	3.2×10^{8}	$>2.0\times10^{8}$	5.3×10^{8}	$<10^{4}$	$<10^{3}$
			3	-	2.0×10^{8}	$>2.0\times10^{8}$	2.8×10^{8}	$<10^{4}$	$<10^{6}$
	8	Top	1	-	4.5×10^{7}	1.8×10^{4}	1.9×10^{9}	$<10^{3}$	$<10^{2}$
			2	-	10^{5}	1.2×10^{4}	8.3×10^{9}	$>10^{5}$	$<10^{2}$
			3	-	2.0×10^{5}	9.7×10^{3}	8.9×10^{8}	$<10^{4}$	10^{2}
		Middle	1	-	4.5×10^{6}	2.3×10^{4}	1.1×10^{7}	$>10^{6}$	<10
			2	-	2.8×10^{4}	1.3×10^{4}	3.0×10^{7}	-	10
			3	-	4.2×10^{5}	9.4×10^{3}	10^{7}	$<10^{2}$	10
		Bottom	1	-	1.8×10^{5}	1.1×10^{3}	10^{7}	$<10^{5}$	$<10^{5}$
			2	-	1.4×10^{8}	4.6×10^{3}	9.3×10^{7}	$>10^{2}$	$>10^{2}$
			3	-	6.2×10^{7}	9.0×10^{6}	1.4×10^{10}	$<10^{3}$	10^{3}

XI (7) EXPERIMENT 02 - TABLE A7

Percentage DM

Treatment		A		B		C		D	
Silo		1	2	3	4	5	6	7	8
Sample									
Top	1	15.8	16.0	18.5	17.9	15.5	15.2	12.1	13.1
	2	15.4	16.3	19.0	17.9	15.4	15.8	13.9	12.6
	3	16.1	16.0	19.5	18.6	15.1	15.8	14.1	14.4
Middle	1	16.9	17.0	18.1	19.6	15.6	16.0	15.7	15.2
	2	16.7	19.3	17.7	17.1	17.5	16.8	17.7	17.0
	3	16.7	15.7	18.3	17.2	16.4	17.1	17.2	17.1
Bottom	1	16.8	16.9	15.0	11.7	13.5	15.3	16.1	13.8
	2	18.9	17.1	16.6	20.1	16.0	16.5	18.2	17.0
	3	18.3	18.3	15.8	14.7	15.5	17.0	17.2	17.6

XI (8) EXPERIMENT 02 - TABLE A8

pH

Treatment		A		B		C		D	
Silo		1	2	3	4	5	6	7	8
Sample									
Top	1	4.88	4.31	5.48	6.00	4.68	4.83	8.26	8.22
	2	4.80	4.19	5.58	5.80	4.77	4.73	8.46	8.45
	3	4.60	4.19	5.49	7.40	4.74	4.53	8.43	8.31
Middle	1	4.81	4.21	5.39	5.80	4.32	4.36	7.11	8.38
	2	4.70	4.78	5.51	5.28	4.23	4.22	4.33	4.32
	3	4.80	5.21	5.22	5.06	4.23	4.22	4.34	4.28
Bottom	1	4.66	3.90	5.41	5.05	4.32	4.41	5.05	7.97
	2	4.01	3.90	5.12	4.45	4.24	4.21	4.35	4.30
	3	4.02	3.92	5.34	4.11	4.21	4.24	4.28	4.25

XI (9) EXPERIMENT 02 - TABLE A9

Percentage WSC in DM.

Treatment		A		B		C		D	
Silo		1	2	3	4	5	6	7	8
Sample									
Top	1	0.2	0.5	0.4	0.6	4.6	6.0	1.4	0.5
	2	0.4	0.5	0.4	1.0	6.7	6.1	1.4	0.6
	3	0.3	0.5	0.1	0.6	6.0	2.6	1.2	1.4
Middle	1	0.3	0.7	0.3	0.5	1.3	2.9	2.8	1.1
	2	0.4	0.7	0.6	0.8	1.6	1.6	1.9	2.2
	3	0.4	0.3	0.7	0.7	1.2	2.8	2.1	1.7

EXPERIMENT 02 - TABLE A9 (contd.)

Treatment		A		B		C		D	
Silo		1	2	3	4	5	6	7	8
Sample									
Bottom	1	0.4	1.2	0.2	1.4	1.6	4.4	0.8	0.8
	2	0.6	0.8	0.9	0.9	1.5	1.5	1.7	1.6
	3	0.6	0.9	0.7	0.9	1.4	1.8	2.5	1.3

XI (10)

EXPERIMENT 02 - TABLE A 10

Percentage MAD-F in DM

Treatment		A		B		C		D	
Silo		1	2	3	4	5	6	7	8
Sample									
Top	1	34.4	32.4	40.1	45.8	28.7	29.5	36.8	38.8
	2	35.8	31.9	39.5	36.7	27.8	30.1	36.1	38.8
	3	36.3	31.5	39.2	41.5	28.2	31.1	37.6	40.9
Middle	1	32.9	34.2	41.5	39.1	28.2	29.8	32.6	36.9
	2	34.0	34.0	34.9	34.9	30.2	31.6	29.0	28.2
	3	35.9	36.5	33.4	33.1	29.8	31.3	29.1	29.9
Bottom	1	30.8	31.1	36.3	31.2	26.5	30.5	30.6	36.6
	2	30.2	32.4	31.8	30.9	29.4	31.4	30.5	28.1
	3	31.8	32.9	33.4	28.5	27.9	31.6	28.1	29.1

XI (11) EXPERIMENT 02 - TABLE A 11.

Percentage organic acids in DM.

Treatment	Silo	Sample	Acetic	Propionic	Butyric	Lactic	Succinic
A	1	Top 2	10.5	0.7	0.3	nil	nil
		Bottom 2	5.9	0.3	0.1	13.0	tr
	2	Top 2	7.5	0.3	0.3	4.9	nil
		Bottom 2	1.4	0.2	0.4	12.6	0.3
B	3	Top 2	10.1	1.0	1.4	nil	0.2
		Bottom 2	10.3	1.2	0.5	4.0	0.4
	4	Top 2	10.2	1.0	1.8	nil	nil
		Bottom 2	5.1	0.4	0.8	9.9	0.4
C	5	Top 2	0.9	0.1	0.3	1.3	tr
		Bottom 2	2.1	0.1	0.1	6.9	0.6
	6	Top 2	1.3	0.1	tr	4.7	0.4
		Bottom 2	1.3	0.2	0.2	6.8	0.6
D	7	Top 2	0.2	0.2	0.3	tr	nil
		Bottom 2	1.6	0.4	0.5	5.9	0.2
	8	Top 2	0.5	1.2	0.9	0.7	0.4
		Bottom 2	1.4	0.1	0.1	10.1	0.7

XI (12) EXPERIMENT 02 - TABLE A 12.

Microbial assay of individual silage samples.
(counts/10g silage)

Treatment	Silo	Sample		VRBA	TAA	MEA	YEA	LF	PC
A	1	Top	1	-	6.8×10^{8}	2.1×10^{4}(M)	2.3×10^{8}	10	10^{2}
			2	-					
			3	-	3.5×10^{9}	2.0×10^{2}	1.1×10^{9}	10	10^{2}
		Middle	1	-	9.7×10^{8}	10^{2}	10^{9}	<10	10^{2}
			2	-	8.1×10^{8}	10^{2}	3.9×10^{8}	<10	10
			3	-	6.6×10^{8}	10^{2}	3.5×10^{8}	<10	10
		Bottom	1	-	7.4×10^{8}	9.2×10^{3}	3.0×10^{7}	<10	10^{2}
			2	-	2.2×10^{8}	10^{2}	1.7×10^{8}	<10	10^{2}
			3	-					
	2	Top	1	<10^{2}	6.1×10^{8}	10^{2}	3.7×10^{8}	<10	10
			2	"	7.7×10^{8}	10^{2}	7.5×10^{8}	<10	10
			3	"					
		Middle	1	"	1.7×10^{9}	10^{2}	9.5×10^{8}	<10	10
			2	"	1.6×10^{9}	10^{2}	9.4×10^{8}	10^{3}	10
			3	"	9.8×10^{7}	10^{2}	5.4×10^{7}	10^{4}	10
		Bottom	1	"	7.4×10^{6}	1.1×10^{5}	5.0×10^{6}	<10	10
			2	"					
			3	"	4.0×10^{7}	3.3×10^{3}	1.3×10^{7}	10	10
B	3	Top	1	-	1.3×10^{8}	10^{2}	4.6×10^{8}	10^{4}	10^{4}
			3	-					
			2	-	1.9×10^{8}	10^{2}	1.1×10^{9}	10	10^{3}
		Middle	1	-	4.0×10^{8}	10^{2}	8.5×10^{8}	<10	10^{2}
			2	-	2.1×10^{8}	10^{2}	3.9×10^{8}	10^{3}	10^{3}
			3	-	4.3×10^{8}	2.0×10^{2}	4.6×10^{8}	10^{2}	10^{3}

EXPERIMENT 02 - TABLE A 12 (contd.)

Treatment	Silo	Sample		VRBA	TAA	MEA	YEA	LF	PC
B	3	Bottom	1	-	1.7×10^8	1.2×10^4	4.7×10^8	<10	10^3
			2	-	3.5×10^8	10^2(M)	1.9×10^8	10^2	10^2
			3	-	6.2×10^8	10^2(M)	3.1×10^8	10^2	10
	4	Top	1	-	1.9×10^9	5.8×10^7	1.6×10^9	<10	10
			2	-	2.6×10^9	4.0×10^6	1.8×10^9	<10	10^3
			3	-	3.7×10^9	-	2.1×10^9	<10	10^2
		Middle	1	-	1.0×10^9	2.6×10^7	7.2×10^9	<10	10^4
			2	-	5.0×10^7	10^4(M)	1.5×10^8	10	10^3
			3	-	3.9×10^7	10^4(M)	3.8×10^7	10^2	10^2
		Bottom	1	-	4.9×10^8	1.5×10^3	5.2×10^8	10^5	10^6
			2	-	2.0×10^8	3.0×10^3	2.1×10^8	<10	10^3
			3	-	1.4×10^7	3.0×10^4	1.3×10^7	<10	10
C	5	Top	1	-	5.4×10^7	5.0x10	4.7×10^8	<10	$<10^4$
			2	-	6.1×10^7	5.0x10	7.0×10^8	<10	$<10^4$
			3	-	6.3×10^7	10	6.8×10^8	<10	$<10^3$
		Middle	1	-	7.9×10^8	10	9.3×10^8	<10	$<10^2$
			2	-	4.0×10^7	5.0x10	4.1×10^8	$<10^2$	$<10^2$
			3	-	2.1×10^7	2.5×10^2	4.6×10^8	<10	$<10^2$
		Bottom	1	-	1.9×10^8	10^3	2.1×10^8	<10	<10
			2	-	4.0×10^8	4.0×10^3	3.7×10^8	<10	<10
			3	-	4.1×10^8	2.1×10^4	9.4×10^8	<10	<10
	6	Top	1	-	6.7×10^8	10	3.6×10^8	<10	<10
			2	-	1.4×10^9	10	5.5×10^8	<10	<10
			3	-	7.9×10^8	2.0x10	3.7×10^8	<10	<10
		Middle	1	-	2.8×10^8	4.0×10^2	1.7×10^8	$<10^2$	$<10^2$

EXPERIMENT 02 - TABLE A 12 (contd.)

Treatment	Silo	Sample		VRBA	TAA	MEA	YEA	LF	PC
C	6	Middle	2	-	$1.1x10^9$	3.0x10	$7.6x10^8$	$<10^2$	$<10^2$
			3	-	$9.7x10^8$	10	$8.1x10^8$	$<10^2$	$<10^2$
		Bottom	1	-	$3.0x10^8$	$7.5x10^2$	$2.6x10^8$	<10	<10
			2	-	$1.3x10^9$	$5.5x10^2$	$9.3x10^8$	<10	<10
			3	-	$6.4x10^8$	$1.7x10^4$	$4.7x10^8$	<10	<10
	7	Top	1	-	$1.8x10^7$	10	$1.2x10^8$	<10	$<10^3$
			2	-	$2.0x10^7$	10	$1.8x10^8$	$<10^3$	$<10^3$
			3	-	$3.7x10^7$	10	$1.2x10^8$	$<10^3$	$<10^3$
		Middle	1	-	$1.1x10^7$	10	$3.9x10^7$	$<10^2$	$<10^2$
			2	-	$2.0x10^8$	5.0x10	$1.7x10^8$	$<10^4$	$<10^3$
			3	-	$1.9x10^8$	2.0x10	$1.7x10^8$	$<10^4$	$<10^3$
		Bottom	1	-	$8.5x10^7$	10	$1.2x10^8$	$<10^2$	$<10^4$
			2	-	$8.0x10^7$	10	$1.1x10^8$	$<10^2$	$<10^4$
			3	-	$9.0x10^7$	2.0x10	$1.2x10^8$	$<10^2$	$<10^4$
	8	Top	1	-	$2.0x10^8$	4.0x10	$1.7x10^8$	$<10^3$	$<10^2$
			2	-	-	-	-	-	-
			3	-	$4.7x10^8$	10	$2.9x10^8$	$<10^3$	$<10^2$
		Middle	1	-	$5.0x10^8$	10^3	$4.0x10^8$	<10	$<10^2$
			2	-	$2.1x10^8$	2.0x10	$1.5x10^8$	$<10^3$	$<10^2$
			3	-	$2.0x10^8$	10	$2.4x10^8$	$<10^3$	$<10^2$
		Bottom	1	-	$2.7x10^8$	$2.5x10^2$	$1.4x10^9$	<10	<10
			2	-	$3.0x10^8$	4.0x10	$3.7x10^8$	<10	$<10^2$
			3	-	$3.5x10^8$	10	$4.0x10^8$	<10	$<10^2$

(M) - Moulds.

XI (13) EXPERIMENT F6 - TABLE A 13.

pH value of test-tube silages.

Treatment		Fresh	Fresh & formic acid	Wilted	Wilted & formic acid
Day	1	5.43	4.92	5.50	5.68
		4.37	4.88	5.60	5.28
	3	4.32	4.50	4.82	5.28
		4.31	4.60	4.86	5.20
	8	4.11	4.42	4.58	4.85
		4.14	4.30	4.60	4.72
	28	4.02	3.98	4.32	4.28
		4.01	4.02	4.33	4.31
	70	4.11	4.10	4.24	4.21
		4.10	4.09	4.28	4.20
	171	3.91	4.00	4.27	4.21
		3.90	3.98	4.20	4.21

XI (14) EXPERIMENT F6 - TABLE A 14.

WSC content of test-tube silages (Per cent DM ensiled).

Treatment		Fresh	Fresh & formic acid	Wilted	Wilted & formic acid
Day	1	15.3	18.4	16.3	16.4
		15.2	19.6	16.4	17.1
	3	5.8	15.8	11.6	16.4
		5.3	18.7	11.8	19.8
	8	4.2	12.9	7.2	19.0
		4.9	14.0	6.9	15.4
	28	3.1	7.2	4.7	17.4

EXPERIMENT F6 - TABLE A 14. (contd.)

Treatment		Fresh	Fresh & formic acid	Wilted	Wilted & formic acid
Day	28	3.8	9.9	5.0	15.3
	70	3.4	5.5	3.8	14.0
		4.8	6.8	3.8	10.5
	171	2.6	4.8	4.1	14.5
		2.4	5.4	3.2	14.1

XI (15) EXPERIMENT F6 - TABLE A 15.

Ethanol content of test-tube silages (Per cent DM ensiled)

Treatment		Fresh	Fresh & formic acid	Wilted	Wilted & formic acid
Day	8	0.71	0.38	0.40	0.28
		0.71	0.55	0.47	0.58
	28	0.81	1.42	0.56	0.40
		0.89	1.70	0.59	0.64
	70	0.82	2.36	0.59	1.29
		0.88	2.25	0.75	2.00
	171	0.80	2.93	0.47	0.80
		0.74	2.90	0.72	0.81

XII. PUBLISHED PAPERS.

Effect of Formic Acid on the Fermentation of Grass of Low Dry Matter Content

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Three experiments are described in which formic acid was added at different levels to low dry matter herbages. In the first and second experiments, using timothy/meadow fescue, the levels of acid used were 0·22% and 0·34% respectively, whereas in the third experiment, using cocksfoot, the concentration of acid was 0·51%. The immediate effect of the formic acid was to lower the pH values to 4·75, 3·81 and 4·11 in experiments 1, 2 and 3 respectively. Formic acid preserved water-soluble carbohydrates in the early stages of harvesting and ensiling but did not prevent the formation of lactic acid in experiments 1 and 3. Ethanol concentrations in the formic acid-treated materials in the first two experiments were higher than in the control silages. In all three experiments, the addition of formic acid did not reduce loss of dry matter and, in experiment 2, losses from the formic acid-treated herbage were higher than those from the control materials. This can be attributed mainly to the higher effluent production when formic acid was used. Changes in carbohydrates, nitrogenous components, organic acids and digestible nutrients are described.

Introduction

THE use of formic acid as a silage additive is not new; its use was advocated by Dirks[1] as early as 1926. A few years later, Von Kapff[2] described the 'silo–acid' process in which a mixture of formic and hydrochloric acids was sprinkled over the herbage. More recently, Saue & Breirem[3] have reviewed experiments, carried out in Europe during the period 1940–1960, in which the efficiency of formic acid as a silage additive was compared with that of mineral acids (A.I.V. process). Although, in most experiments, formic acid compared well with A.I.V. acids with respect to its effect on silage quality, extensive examination by the Norwegian workers of silage samples collected at farms gave varying results.

With the introduction of the flail-type forage harvester, the efficiency of formic acid as a silage additive was increased.[3] The process of applying the acid was not simplified, however, until Aas *et al.*[4] devised equipment which could be attached to the forage harvester and which sprayed concentrated formic acid directly on to the grass immediately after cutting.

Although formic acid has been widely used as an additive in Norway and in some other European countries for a number of years, it was not available in the U.K. on any commercial scale until 1968 when the proprietary product 'Add-F'* came on the market. Add-F contains 85% formic acid and the recommended rate of addition is 0·5 gal/ton (2·21 ml/kg) fresh grass equivalent to a concentration of 0·265%. The acid is applied directly on to the herbage from a plastic container attached to the forage harvester.

Apart from the beneficial effects of formic acid on the fermentation,[5] there are several reports[6–8] suggesting that formic acid-treated silage results in better performance when fed to dairy cows and fattening animals.

Because of the increasing interest in the use of formic acid in silage-making, a series of experiments were carried out in which detailed chemical changes and losses were made. The results presented in this paper refer to three experiments carried out with low dry matter herbage of relatively low water-soluble carbohydrate (*WSC*) content using formic acid in the form of Add-F at three different levels.

Experimental

The silo unit used has been described in detail in an earlier publication;[9] it consists of four metal tower silos, each having a capacity of about 1000 kg fresh herbage and each being suspended from a weighing device which enables direct measurement of weight changes. In the first and second experiments, conditions of filling were similar to those reported earlier.[9,10] Consolidation was achieved by tramping the herbage, covering it with plastic sheeting and placing stone blocks (500 kg), corresponding to a pressure of 27·5 g/cm², on the surface. In the third experiment, an attempt was made to simulate conditions on the surface of a farm silo and the herbage was not consolidated with stone blocks, but only covered with plastic sheeting. The formic acid was applied to the grass in the form of Add-F from a polyethylene container attached to the forage harvester. A nozzle was selected to deliver the acid at a suitable rate. By weighing the additive in the container before and after application, the weight of Add-F on a known weight of grass was calculated.

In experiment 1, the grass was pre-treated with a compound fertiliser (23% N, 5% P, 9% K) applied at the rate of 314 kg/ha on both 14 April and 29 April, 1968. On 4 June, 1968, the grass, of composition timothy (*Phleum pratensis*) 69% : meadow fescue (*Festuca pratensis*) 26% : perennial ryegrass (*Lolium perenne*) 5%, was cut with a flail-type forage harvester and ensiled immediately with the following treatments: silo A, 1198 kg grass; silo B, 1198 kg grass; silo C, 1164 kg grass containing 0·22% formic acid; and silo D, 1164 kg grass containing 0·22% formic acid. The silos were opened 63 days after being filled.

In experiment 2, the autumn cut of grass used was taken from the same field as that used in the first experiment and had previously received a dressing of commercial ammonium nitrate (34·5% N), on 16 August, 1968, at the rate of 292 kg/ha. On 23 September, 1968, the grass (meadow fescue 66% : perennial ryegrass 25% : timothy 6% : others 3%) was cut with a flail-type forage harvester and ensiled immediately with the following treatments: silo A, 1261 kg grass: silo B, 1261 kg grass; silo C, 1382 kg grass containing 0·34% formic acid; and silo D, 1382 kg grass containing 0·34% formic acid. The silos were opened 125 days after filling.

In experiment 3, cocksfoot grass (*Dactylis glomerata*) which had received 544 kg/ha of a compound fertiliser (23% N, 5% P, 9% K), applied on 9 April 1969, was cut with a flail-type forage harvester on 27 May, 1969, and ensiled immediately with the following treatments: silo A, 1028 kg grass; silo B, 1028 kg grass; silo C, 1028 kg grass containing 0·51% formic acid; and silo D, 1028 kg grass containing 0·51% formic acid. The silos were opened 163 days after being filled.

Temperature measurements were recorded by thermocouples buried at different levels throughout the herbage.

* Manufactured by Direct Nitrogen Ltd., BP Chemicals Group.

Ambient temperatures were recorded by two thermocouples suspended above each silo. Methods of sampling and analysis of the grasses and silages have been described in earlier publications.[9,11] In addition, nitrate determinations were carried out using the method of ap Griffith & Johnston.[12] Effluents were removed daily, weighed and pH values were recorded. Subsamples were collected and deep frozen, and a bulked sample was analysed at the end of the experiment for true dry matter, ash, nitrogen, sugars, volatile acids and ethanol. Production of effluent was deliberately restricted in all three experiments in order to avoid excessive loss of formic acid, particularly in the early stages of ensilage. In the first and second experiments, free drainage was allowed after 14 and 7 days, respectively. In the third experiment, free drainage was allowed after 14 days from the control silos but the flow of effluent from the treated silages was restricted for 93 days to give similar volumes from all silages.

In addition to the tower silos, laboratory test-tube silos (capacity 80 g) were filled with similar herbages. These laboratory silos were fitted with mercury seals and were opened at intervals throughout the ensiling period, the whole contents being used for analysis.

In experiments 1 and 2, digestibility and intake of these silages were determined with Cheviot wether sheep, using methods described earlier.[9]

Results

Experiment 1

Temperature

There was an immediate rise in temperature in the control silos; 19°C was recorded on the 3rd day. The temperature of the treated silages remained constant (16°C) until the 8th day. There was then a gradual rise until, on the 14th day, maximum temperatures of 20°C in the control silages and 21°C in the acid-treated silages were attained.

Composition

The compositions of the grasses and silages are shown in Table I. The *WSC* value of the formic acid-treated grass was higher than that of the control material. Nitrate-nitrogen contents of the grasses were high and are, presumably, a reflection of the heavy dressing of nitrogenous fertiliser. All silages had a low *WSC* content and contained lactic acid. Silage A had the lowest pH (4·30), corresponding to the highest lactic acid content (10·0%). Silage B contained only 5·1% lactic acid which is reflected in the higher pH value (4·72). Ethanol contents of the control silages were less than half those of the treated silages.

It can be seen in Fig. 1 that the pH of the effluents from the control silages fell below those from the treated silages on the 3rd day and remained lower throughout the experiment.

Losses

The weights (kg) of fresh silage removed were: A, 922; B, 929; C, 893; D, 889. Detailed dry matter losses are given in Table II and some individual component losses in Table III. There were no marked differences between treatments in this experiment.

Digestibility and intake

The digestibility coefficients, digestible nutrients and dry matter intakes of the silages are shown in Table IV. There

TABLE I

Composition of grasses and silages
% of true dry matter

	Experiment 1						Experiment 2					
	Grasses		Silages				Grasses		Silages			
	AB	CD	A	B	C	D	AB	CD	A	B	C	D
Dry matter (*DM*)	13·70	14·53	15·37	15·05	15·90	16·49	12·03	11·84	13·06	13·01	15·64	14·70
Organic matter	89·6	91·7	92·0	88·1	90·6	90·9	89·8	90·0	90·1	90·0	90·8	89·5
Crude protein	23·1	23·4	21·3	22·5	22·7	23·0	26·1	25·6	24·8	25·0	24·2	23·9
Ether extract	2·9	3·0	4·9	4·5	4·4	4·3	3·6	3·5	4·9	5·0	4·5	4·3
Crude fibre	24·4	24·5	27·0	25·8	27·2	27·8	22·2	22·2	22·1	22·5	24·3	24·3
Total N	3·70	3·74	3·42	3·59	3·63	3·67	4·18	4·09	3·97	4·00	3·88	3·83
Protein N	2·81	2·80	1·16	1·38	1·49	1·70	3·12	2·98	1·77	1·74	1·92	2·02
Non-protein N	0·89	0·94	2·26	2·21	2·14	1·97	1·06	1·11	2·20	2·26	1·96	1·81
Volatile N	0·04	0·04	0·32	0·51	0·30	0·35	—	—	0·37	0·33	0·18	0·29
Volatile N as % total N	1·1	1·1	9·4	14·2	8·3	9·5	—	—	9·3	8·3	4·6	7·6
Nitrate N	0·23	0·26	0·14	0·07	0·16	0·14	0·33	0·34	0·30	0·31	0·27	0·25
Protein N as % total N	75·9	74·9	33·9	38·4	41·0	46·3	74·6	72·9	44·6	43·5	49·5	52·7
Water-soluble carbohydrates	8·3	11·9	0·7	0·6	0·8	0·6	7·5	9·5	2·4	2·2	6·3	6·1
Glucose	2·7	3·4	nil	nil	nil	nil	1·1	2·0	0·2	0·2	1·2	1·2
Fructose	3·0	4·0	nil	tr.	nil	nil	1·6	2·9	0·1	0·1	1·7	1·3
Xylose	nil	nil	tr.	tr.	tr.	tr.	nil	nil	0·1	0·3	0·8	0·7
Galactose	nil	nil	tr.	tr.	tr.	tr.	nil	nil	0·3	0·2	0·4	0·4
Arabinose	nil	nil	tr.	tr.	tr.	tr.	nil	nil	tr.	tr.	0·4	0·6
Oligosaccharides (including sucrose)	1·2	1·9	0·2	0·1	0·2	0·1	2·3	1·7	1·3	1·2	0·6	0·6
Fructosans	1·9	2·1	tr.	tr.	tr.	tr.	2·0	2·1	nil	nil	0·3	0·3
Mannitol	nil	nil	0·1	0·1	0·2	0·4	nil	nil	tr.	tr.	nil	nil
Cellulose	27·9	27·4	30·7	28·5	30·6	30·1	25·7	26·0	25·7	26·5	27·5	28·2
Lignin	3·3	3·3	3·7	3·6	3·7	3·7	3·6	3·5	3·7	3·6	4·3	4·5
Formic acid	nil	1·5	nil	nil	1·4	1·4	nil	2·8	nil	nil	0·4	0·5
Acetic acid	nil	nil	6·1	7·5	4·9	4·5	nil	nil	1·2	1·7	1·2	0·8
Propionic acid	nil	nil	nil	nil	0·1	0·2	nil	nil	tr.	tr.	tr.	tr.
Butyric acid	nil	nil	nil	tr.	nil	nil	nil	nil	tr.	tr.	tr.	tr.
Lactic acid	nil	nil	10·0	5·1	5·6	4·1	nil	nil	8·2	7·4	nil	nil
Succinic acid	nil	nil	nil	nil	nil	nil	nil	nil	nil	nil	nil	nil
Ethanol	nil	nil	1·1	1·3	2·5	2·7	nil	nil	0·2	0·4	1·5	2·1
pH after maceration	6·00	4·75	4·30	4·72	4·36	4·42	5·99	3·81	4·01	4·06	4·26	4·40
Buffering capacity, mequiv./100g *DM*	30	34	105	114	96	88	25	49	98	98	51	41

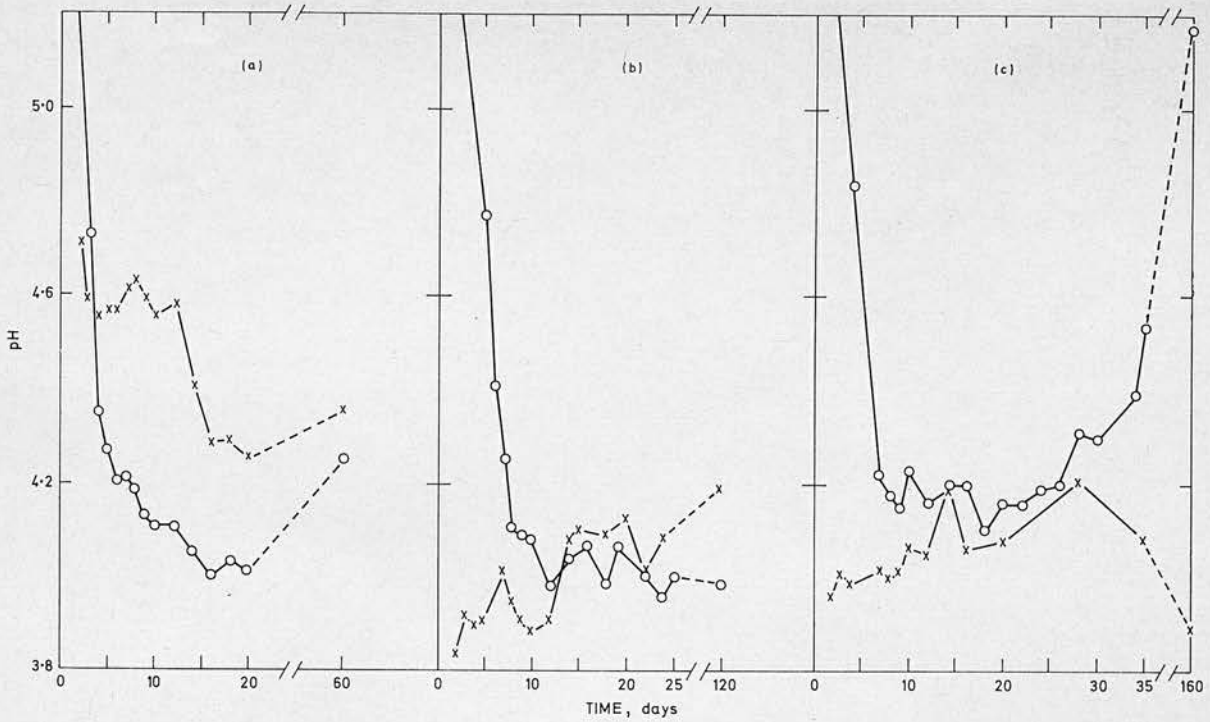

FIG. 1. *Change in effluent pH with time*

(a) Experiment 1; (b) experiment 2; (c) experiment 3; ○ control; × acid-treated

TABLE II

% Loss of dry matter

	Silo			
	A	B	C	D
Experiment 1:				
Fermentation and oxidation	5·0	6·5	9·0	5·6
Effluent	8·6	8·5	7·0	7·1
Waste silage	1·9	2·7	2·2	1·6
Total	15·5	17·7	18·2	14·3
Experiment 2:				
Fermentation and oxidation	1·8	nil	4·4	5·9
Effluent	5·7	4·4	12·0	10·7
Waste silage	nil	nil	3·0	0·5
Total	7·5	4·4	19·4	17·1
Experiment 3:				
Fermentation and oxidation	18·8	16·4	14·8	1·4
Effluent	7·7	12·1	12·1	11·9
Waste silage	27·5	27·5	33·1	40·5
Total	54·0	56·0	60·0	53·8

TABLE III

% Losses of dry matter components

	Silo							
	A		B		C		D	
	Total	Effluent	Total	Effluent	Total	Effluent	Total	Effluent
Experiment 1:								
Organic matter	11·4	7·9	16·4	7·8	17·0	6·0	14·2	6·1
Water-soluble carbohydrates	92·8	7·4	93·5	6·8	94·3	7·3	95·9	7·3
Formic acid	—	—	—	—	22·9	26·4	20·5	26·4
Experiment 2:								
Organic matter	7·3	5·1	4·0	4·0	16·0	10·5	17·1	9·4
Water-soluble carbohydrates	71·1	7·0	71·9	6·1	44·5	41·3	46·5	34·8
Formic acid	—	—	—	—	88·1	37·6	85·3	33·3
Experiment 3:								
Organic matter	36·5	7·6	42·3	11·3	40·9	12·8	40·4	13·1
Water-soluble carbohydrates	98·4	3·9	98·8	4·2	90·7	42·3	90·2	43·1
Formic acid	—	—	—	—	83·5	32·7	79·7	33·5

TABLE IV(a)

% Digestibility (*D*), % of digestible nutrients (*DN*), and energy values of true dry matter

	Experiment 1								Experiment 2							
	A		B		C		D		A		B		C		D	
	D	*DN*	*D*	*DN*	*D*	*DN*	*D*	*DN*	*D*	*DN*	*D*	*DN*	*D*	*DN*	*D*	*DN*
Organic matter	80·0	73·6	79·6	70·1	82·6	74·8	79·8	72·5	77·1	69·4	78·8	70·9	72·5	65·8	75·8	67·8
Crude protein	80·4	17·1	82·4	18·6	83·7	19·0	82·1	18·9	79·4	19·7	82·3	20·6	75·8	18·4	77·5	18·6
Ether extract	76·7	3·8	77·1	3·5	75·2	3·3	72·1	3·1	69·5	3·4	72·4	3·6	54·5	2·5	60·0	2·6
Crude fibre	81·8	22·1	82·2	21·2	86·0	23·4	82·8	23·0	77·4	17·1	77·0	17·4	71·2	17·3	76·8	18·7
N-free extractives	78·9	30·6	76·2	26·9	79·2	27·2	77·0	27·6	76·4	29·2	78·4	29·4	73·3	27·6	76·0	28·1
Starch equivalent		68·3		64·8		66·9		66·2		64·9		66·4		59·9		62·0
Total digestible nutrients		78·4		74·6		77·0		76·5		73·7		75·4		68·9		71·1
Metabolisable energy*		2·99		2·87		3·08		2·98		2·45		2·65		2·28		2·52

* Calculated from calorific values

TABLE IV (b)

Dry matter intakes of the silages, g dry matter/kg $W^{0.73}$

Animal	Experiment 1				Experiment 2			
	A	B	C	D	A	B	C	D
1	53·1	41·3	33·4	67·0	49·7	46·2	41·5	35·9
2	33·1	47·0	43·9	54·8	37·8	35·3	33·7	45·5
Mean	43·1	44·2	38·7	60·9	43·8	40·8	37·6	40·7

were no significant statistical differences in values for the treated and untreated silages.

Test-tube silos

Changes in pH and *WSC* contents during ensilage were examined by opening tubes at intervals throughout the period; the results are shown in Fig. 2. After an initial rise, the *WSC* content of the control silages fell rapidly, with a similar fall in pH value. After remaining steady during the first few weeks, the pH rose to a value similar to that of control silage B. The pH values of the test-tube-treated silages fell gradually to pH 4·0 and then remained constant until the end of the experiment.

Experiment 2

Volume

Herbage in the control silos was tramped to a volume of 3·02 m^3 before plastic sheeting and stones were placed on the surface. Similar tramping to that in silos A and B resulted in a smaller initial volume (2·20 m^3) in silos C and D. By the 3rd day, the volumes of the control and treated materials had decreased to 1·56 m^3 and 1·47 m^3, respectively, and at the end of the ensiling period they were 1·26 m^3 and 1·14 m^3.

Temperature

The highest mean temperature (14°C) in the control silages was recorded on the 2nd, 4th and 5th days. By the 9th day, the mass had reached ambient temperature and recordings were not made after the 11th day. Temperatures in the treated silages remained at, or just below, ambient temperature throughout the 11 days.

Composition

Compositions of the grasses and silages are shown in Table I. The higher *WSC* content of the treated grasses illustrates again the effect formic acid has in preserving these fermentable carbohydrates. The residual sugars were high in the control silages and exceptionally high in the treated silages. Formic acid values for the treated silages were low and suggest a breakdown of formic acid during ensilage Ethanol values were again higher for the treated silages. The nitrate-nitrogen content of the grasses was exceptionally high and the recovery of this nitrate in the silages was surprising. The control silages had a higher volatile-nitrogen content than the treated silages. The pH values of the effluents (Fig. 1) from the control silages fell rapidly during the first ten days and then remained fairly constant (around pH 4·0) throughout the experiment. The pH values of the effluents from the treated silages rose from 3·84 to 4·02 in the first 7 days, decreased to 3·88 with the increase in effluent flow and rose again to 4·10 by the 16th day. The final pH value of the effluent was 4·2.

Losses

The weights (kg) of fresh silage removed were: A, 1075; B, 1116; C, 876; D, 928. Losses of dry matter and other components are shown in Tables II and III. Losses of dry matter from the treated herbages were higher than from the controls, the effluent differences being particularly large. Losses of *WSC* and formic acid in the effluents from the treated materials were high.

Digestibility and intake

Digestibility coefficients and digestible nutrients for the silages are shown in Table IV. The lower digestibility values for the treated silages can probably be attributed to the higher loss of digestible nutrients in the effluents from these materials. The mean daily dry matter intakes (g/kg $W^{0.73}$) over a period of 30 days are also shown in Table IV. There is no significant difference between the intake figures of the treated and untreated silages.

Test-tube silos

The *WSC* of the control materials followed a similar pattern to that reported in the previous experiment (Fig. 2). In the formic acid-treated herbage, however, the *WSC* content was maintained up to the 37th day but fell to less than 2% by the 142nd day.

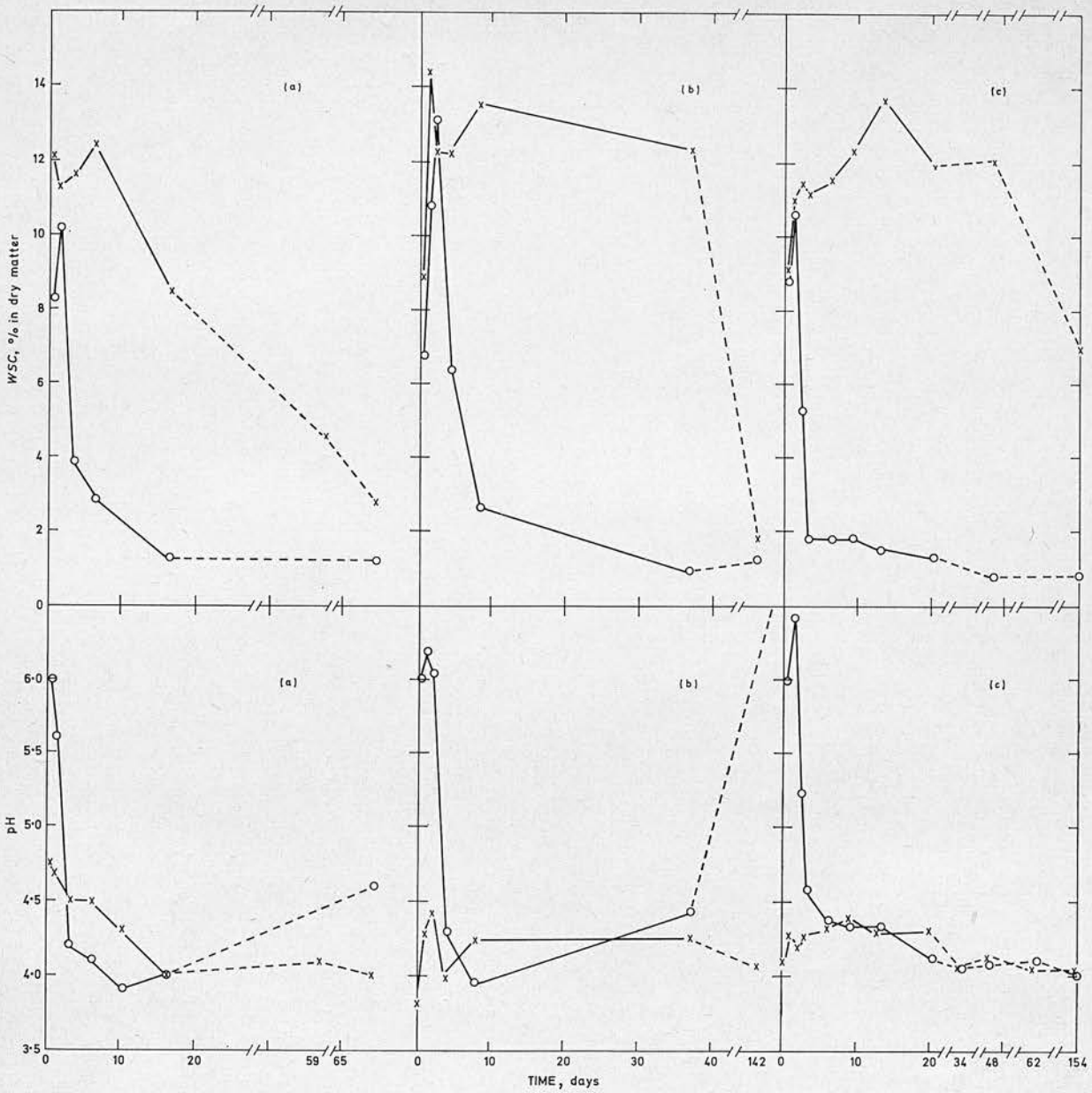

FIG. 2. *pH and* WSC *values for laboratory tube silos*
(a) Experiment 1; (b) experiment 2; (c) experiment 3; ○ control; × acid-treated

Experiment 3

Volume

The initial volumes of the control and treated herbages were 2·57 m³ and 1·70 m³, respectively. The volume of the control material fell rapidly and by the 4th day the corresponding volumes were 1·70 m³ and 1·47 m³, respectively. The silage levels fell throughout the ensiling period and the final volumes were similar (0·83 m³).

Temperature

The maximum mean temperature (29°C) of the control silages was recorded on the 4th day and had fallen to 21°C by the 10th day. The temperature of the treated silages fell during the first few days to 13°C and then rose, at first slowly, and later rapidly to a maximum value of 33°C on the 21st day.

Composition

The compositions of the grasses and silages are shown in Table V. A separate analysis of each grass was made and the high level of soil contamination is confirmed by the high ash figures of the grass and silages. The edible silages only were analysed for *WSC* and organic acids. The acid-treated silages contained some residual *WSC* and lactic acid in this material. Lactic acid, although present in the effluents from the control silos, was no longer present in the silages at the end of the ensiling period.

The pH values (Fig. 1) of the control effluents fell rapidly during the first few days and remained below 4·20 until the 25th day; the values then increased until the 80th day after which they remained fairly constant. Effluents from the treated silages had an initial pH of 3·96 which rose to 4·08 on the 10th day. There was then only a slight rise in pH to a maximum value of 4·23. On the 93rd day, free drainage was allowed from the silos resulting in a fall in pH of effluents C and D. During the last 50 days of ensilage, the pH values of these effluents fell from 4·03 to 3·89.

TABLE V

Composition of grasses and silages

% of true dry matter

	Experiment 3							
	Grasses				Edible silages			
	A	B	C	D	A	B	C	D
Dry matter (*DM*)	14·64	13·65	16·20	17·30	13·59	16·53	19·84	20·80
Organic matter	76·6	81·1	73·9	71·5	73·2	66·9	68·9	68·0
Crude protein	17·4	18·7	18·7	18·3	18·6	17·3	17·4	16·3
Ether extract	3·9	3·8	3·1	3·2	2·9	2·9	3·1	3·0
Crude fibre	20·9	21·6	19·7	19·0	22·9	22·2	20·3	20·7
Total N	2·78	2·99	2·99	2·92	2·98	2·77	2·78	2·61
Protein N	2·16	2·37	2·52	2·45	1·39	1·16	1·43	1·48
Non-protein N	0·62	0·62	0·47	0·47	1·59	1·61	1·35	1·13
Volatile N	0·02	0·02	0·02	0·02	0·76	0·98	0·45	0·37
Volatile N as % total N	0·7	0·7	0·7	0·7	25·5	35·4	15·9	13·7
Nitrate N	0·11	0·10	0·20	0·20	tr.	tr.	0·09	0·05
Protein N as % total N	77·7	79·3	84·3	83·9	46·6	41·9	51·4	56·7
Water-soluble carbohydrates	10·6	9·3	8·8	9·3	0·4	0·2	2·1	2·3
Glucose	2·1	2·5	2·5	2·9	tr.	tr.	0·2	0·2
Fructose	5·1	4·5	4·2	4·2	nil	nil	0·5	0·5
Xylose	nil	nil	nil	nil	nil	nil	0·3	0·2
Galactose	nil	nil	nil	nil	tr.	tr.	0·3	0·3
Arabinose	nil	nil	nil	nil	nil	nil	nil	0·1
Oligosaccharides (including sucrose)	0·6	0·6	0·3	0·3	tr.	tr.	0·1	0·1
Fructosans	1·7	1·0	1·0	1·1	nil	nil	tr.	tr.
Mannitol	nil	nil	nil	nil	nil	nil	tr.	tr.
Cellulose	22·1	23·8	22·1	20·8	23·9	23·9	23·5	22·3
Lignin	3·9	3·5	3·7	3·3	4·8	5·4	4·6	5·3
Formic acid	nil	nil	3·2	3·0	nil	nil	1·3	1·5
Acetic acid	nil	nil	nil	nil	8·7	6·3	2·7	3·1
Propionic acid	nil	nil	nil	nil	2·0	1·5	0·1	0·3
Butyric acid	nil	nil	nil	nil	4·3	3·3	1·1	1·9
Lactic acid	nil	nil	nil	nil	nil	nil	4·4	3·9
Succinic acid	nil	nil	nil	nil	nil	nil	nil	nil
Ethanol	nil	nil	nil	nil	1·2	1·0	0·7	0·4
pH after maceration	6·01	6·01	4·11	4·11	4·90	4·98	4·19	4·28
Buffering capacity, mequiv./100g *DM*	41	37	40	44	175	169	94	107

Losses

The weights (kg) of fresh silage removed were: A, 717; B, 571; C, 567; D, 581. Detailed dry matter losses given in Table II show that the waste losses were very high in both control and formic acid-treated materials. The fermentation + oxidation loss from silo D is surprisingly low and could have resulted from a sampling error caused by variable soil content throughout the herbage. Because of this, the organic matter values in Table III are more reliable for comparison purposes than are the dry matter values.

Test-tube silos

The *WSC* values in the control and treated materials (Fig. 2) showed a similar pattern to that obtained in experiment 2.

At the end of the experiment, the tube silages had a lower pH value than did those of the main experiment. The acid components of the dry matter at the later stages of ensiling are given in Table VI.

TABLE VI

Organic acids in test-tube silages during later stages of experiment 3

% of true dry matter

Day	pH		Formic acid		Acetic acid		Lactic acid	
	C	T	C	T	C	T	C	T
34	4·08	4·10	nil	3·2	2·4	0·2	12·2	0·2
62	4·13	4·07	nil	2·4	5·5	0·6	10·6	2·3
154	4·03	4·06	nil	1·9	3·5	1·7	11·6	1·7

C = Control
T = Formic acid-treated silage

Discussion

The object in these experiments was to study the effectiveness of formic acid as a silage additive on 'difficult' crops. The grasses used here were low in dry matter (11·8–17·3%), low in *WSC* (7·5–11·9%) and high in nitrogen (2·78–4·18%). In the first experiment, the formic acid was added at the commercially recommended level (0·22% of the fresh herbage), while in the second and third experiments, higher levels of 0·34% and 0·51% respectively were used.

The immediate effects of formic acid were to lower the pH values to 4·75, 3·81 and 4·11 in experiments 1, 2 and 3, respectively. It should be noted that these pH determinations were made on macerates and not on chopped plant material immersed in water, a procedure which leads to erroneously low values. The initial pH of the grass macerate in the third experiment was higher than that of material in the second because the cocksfoot grass used was of higher buffering capacity. In experiment 1, with similar compaction, no differences in initial volumes of silages were noted. In experiments 2 and 3, however, the formic acid-treated grass compacted more readily. At the higher levels of application, formic acid appeared to affect the structure of the grass.

In the first two experiments, formic acid prevented oxidation of the *WSC* in the short period (about 4 h) between harvesting and ensiling, thereby preserving more sugars for fermentation. This finding was not apparent in the third experiment and soil contamination, which appeared to be exceptionally high, may have confused the results. In the test-tube silos, the pattern of *WSC* contents, as seen in Fig. 2, shows a rise in both

control and formic acid-treated materials. Chromatographic analysis has confirmed that this rise is caused by the production of xylose, arabinose and galactose from the hydrolysis of heteropolysaccharides. Where formic acid is used at a high level, the concentration of *WSC* is maintained over a period of weeks before fermentation subsequently occurs.

Formic acid has an inhibiting effect upon proteolytic clostridia, as is shown by the volatile N figures given in Tables I and V; this finding confirms that of other workers.[6,13] Less proteolysis occurred with formic acid-treated herbage. The grasses used in the 3 experiments were all heavily fertilised with nitrogenous fertilisers; the effect of this treatment is shown by the high nitrate-nitrogen figures. Experiment 2 is atypical in that the nitrate was not broken down to any extent during ensilage; this is contrary to the finding of Wieringa[14] that nitrate in concentrations between 0·06 and 0·4% is rapidly reduced in silage.

When formic acid is used at the lowest level (0·22%), it does not prevent the formation of lactic acid and results of the first experiment agree in this respect with those of other workers.[3,6] The results from experiment 2 show that no lactic acid was found, indicating that the lactic acid bacteria had been completely inhibited. The pattern was again different in the third experiment in that a saccharolytic clostridial fermentation had occurred in the large control silos. This was a secondary fermentation, as originally lactic acid had been produced but had subsequently been degraded to butyric acid. Evidence for this was obtained from analysis of the effluents, which contained lactic acid. Although some butyric acid was present in the treated silages, this was much lower than in the control materials, and lactic acid was the major acid present. These differences are reflected in the pH values.

Ethanol is known to be produced during ensilage by the action of heterofermentative lactic acid bacteria on glucose;[11] it can also be formed by the action of yeast organisms on hexoses. In experiments 1 and 2, ethanol concentrations were higher in the formic acid-treated silages than in the control materials and it is clear from the amounts present that a considerable proportion of this alcohol had not resulted from the activities of the heterofermentative lactic acid bacteria. The absence of lactic acid in the treated silages and effluents in experiment 2 substantiates this theory. The possibility of a yeast fermentation in formic acid-treated herbage is being examined.

In considering the losses during ensilage, experiment 3 should be treated separately because of the deliberate intention to encourage aerobiosis. In experiment 1, losses of dry matter and individual nutrients were similar in both treatments. In experiment 2, however, the dry matter losses from the acid-treated herbage were considerably higher than those from the control materials. These losses can be attributed to the higher effluent production and formation of waste. In experiment 3, there were no differences in losses between treatments, the high values obtained being attributable to excessive oxidation. Formic acid did not reduce the amount of surface waste material formed.

In all experiments, considerable amounts (26–38%) of formic acid were lost in the effluents. In experiments 2 and 3, complete recoveries of formic acid were not obtained and it would appear that about half of the formic acid had disappeared during ensilage. The cause of this loss of formic acid is not known, but there is evidence from the laboratory studies in experiment 3 (Table VI) that this loss of acid occurred in the later stages of the fermentation process.

In the high formic acid-treated herbages, over 40% of the *WSC* were lost in the effluent. This is another relevant point against ensiling very wet crops and it is significant that Norwegian workers[8] have recommended that silos without drainge should be used, as in the case of molasses-treated herbage.

The results of the digestibility trials did not indicate any marked differences between treatments. The intake figures do not support the findings of other workers who have reported higher intakes on formic acid-treated silages.[6,7] In the two experiments where feeding trials were carried out, the control silages were well preserved and this could have been an important factor in preventing any differences between treatments occurring.

In conclusion, the use of formic acid as a silage additive on low dry matter grass affects the fermentation pattern, especially when used at high levels. In experiments 1 and 2, formic acid decreased the formation of lactic acid and volatile nitrogen but increased the production of ethanol. In experiment 3, the additive restricted the breakdown of lactic acid to butyric acid and the formation of volatile nitrogen.

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Chemical Changes and Losses during the Ensilage of Wilted Grass Treated with Formic Acid

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Formic acid (85%) was added to wilted perennial ryegrass (36% dry matter) at the rate of 0.39%. Changes during ensilage of this material were compared with changes occurring during ensilage of untreated wilted ryegrass and freshly harvested herbage. All silages were well preserved, of low volatile N content and contained only traces of butyric acid. Formic acid restricted fermentation in the wilted grass resulting in silage of high water-soluble carbohydrate content (15.3%) compared with untreated wilted (4.7%) and fresh (1.2%) silages. Results of microbiological studies indicated that yeasts were more active in the formic acid-treated herbages. Surface waste production and fermentation plus oxidation losses were higher in the acid-treated wilted silages (21%) than in the untreated wilted materials (14%).

1. Introduction

The advantages of wilting crops to a dry matter (d.m.) content of about 30% prior to ensiling have been stressed by several workers.[1–4] Apart from reducing or eliminating effluent losses, wilting restricts or has a selective influence on microbial activity[5,6] and produces a silage more acceptable to ruminants.[4,7] One problem frequently associated with wilted herbage is the difficulty in achieving the degree of consolidation necessary to exclude air from the ensiled mass. Studies in which formic acid was applied to freshly harvested grass indicated that this additive restricted oxidation of the water-soluble carbohydrate (w.s.c.) between harvesting and ensiling.[8] It is possible that formic acid may have a beneficial effect in reducing respiration as well as in restricting microbial activity in ensiled wilted herbage. The results presented in this paper refer to an experiment in which the chemical and microbiological changes were examined in ensiled wilted grass, pretreated with formic acid, and in ensiled wilted herbage.

2. Experimental

The main silo unit in this experiment consisted of four metal silos,[9] each having a maximum capacity of about 1000 kg of fresh herbage and each suspended from a weighing apparatus sufficiently sensitive to record a change in weight in the silo and contents of 0.1 kg.

Perennial ryegrass (*Lolium perenne*) obtained from one of the school farms was cut with a mower on 8 June 1970 at 8 am and wilted for 31 h in the field before being lifted

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with a flail-type forage harvester. Untreated wilted grass was ensiled in silos A and B. The other two metal silos C and D were filled with similar wilted herbage treated with formic acid in the form of "Add-F"[a]. The Add-F was applied to the grass at the rate of 0.39% (equivalent to 0.33% pure formic acid) from a polyethylene container attached to the forage harvester as described in an earlier paper.[8]

The ensiled herbage was covered with polyethylene sheeting and after 48 h delay was consolidated with stone blocks corresponding to a surface pressure of 38 mbar (380 N/m^2). Assessment of true losses and surface waste measurements were made using a bag and marker technique already described.[10] Temperature measurements using eight thermocouples buried at different levels in each silo were recorded daily.

On 8 June 1970, two reinforced 2000 kg capacity PVC silos (E and F), similar to those described by Harris, Raymond and Wilson,[11] were filled with fresh herbage taken from the same field as that used in the wilting experiment. Only chemical changes were studied in these silos, the main object was to provide unwilted grass silage for a subsequent animal experiment. The analytical results for grass and silages are reported in this paper for comparison purposes.

Silos A, B, C, D, E and F were opened 149, 179, 156, 179, 179 and 114 days after filling, respectively.

Methods of sampling, chemical and microbiological analyses of grasses and silages were similar to those reported in earlier publications.[3,9]

In addition to the tower and plastic silos, laboratory test-tube silos (capacity 80 g) were filled with herbages similar to those used in the main experiment. An additional treatment was fresh grass plus Add-F, applied at the rate of 0.27% fresh herbage. The

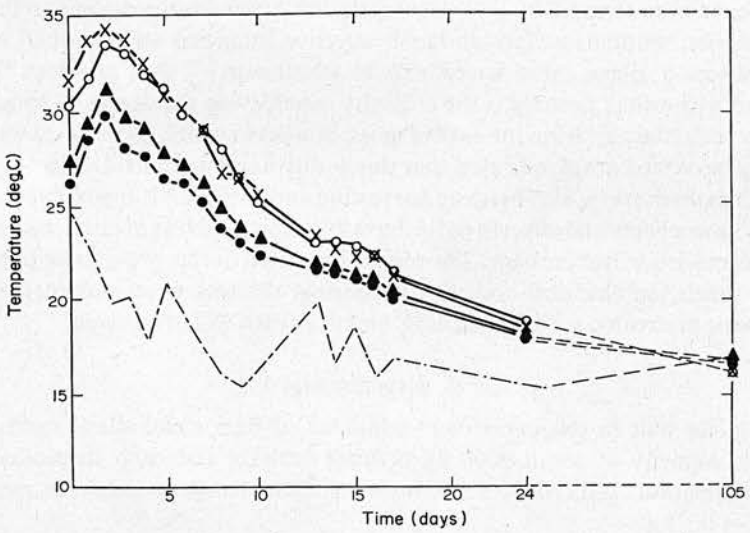

Figure 1. Temperature changes with time.
—×—×—, Silo A; —○—○—, silo B; —▲—▲—, silo C; —●—●—, silo D; - — - —, ambient temperature.

[a] A product containing 85% formic acid and manufactured by BP Chemicals International Ltd.

laboratory silos were fitted with mercury seals and duplicates were opened at intervals throughout the ensiling period. Ethanol, w.s.c. and pH values only are reported for these samples.

3. Results

3.1. Temperature changes

The temperature changes in the four silos are shown in Figure 1, each point on the graph representing the mean of eight thermocouple recordings. The temperatures followed a similar pattern, i.e. an initial rise followed by a decline after consolidation on the third day. Temperatures recorded in the formic acid-treated materials were consistently lower than those obtained in the untreated silages.

TABLE 1. Composition of grasses and silages (% of dry matter)

	Grass			Silage					
	Fresh	Wilted	Wilted and formic	A (W)[a]	B (W)	C (WF)	D (WF)	E (U)	F (U)
Dry matter (d.m.)	17.75	32.33	36.00	30.82	30.90	32.88	34.49	18.04	18.68
Organic matter	93.0	92.8	93.1	92.9	92.0	92.7	92.5	93.1	93.2
Crude protein	14.2	13.4	14.4	14.3	14.0	15.1	15.0	14.6	14.4
Ether extract	2.41	1.94	2.37	2.47	2.56	2.46	2.69	3.5	3.4
Crude fibre	26.5	26.9	25.7	28.7	29.3	28.0	29.5	31.0	29.8
Total N	2.27	2.15	2.31	2.28	2.24	2.42	2.40	2.34	2.30
Protein N	1.84	1.47	1.58	0.66	0.47	0.76	0.63	0.36	0.54
Non-protein N (n.p.n.)	0.43	0.68	0.73	1.62	1.77	1.66	1.77	1.98	1.76
Volatile N	—	—	—	0.19	0.19	0.16	0.16	0.20	0.18
Volatile N as % total N	—	—	—	8.3	8.3	6.8	6.7	8.5	7.8
Water soluble carbohydrates (w.s.c.)	17.7	16.8	18.5	4.75	4.56	15.1	15.4	1.44	0.99
Glucose	4.4	3.1	4.8	1.6	1.5	3.2	4.0	0.3	0.2
Fructose	2.9	3.4	3.9	1.4	0.9	7.5	7.4	0.3	0.3
Xylose	—	—	—	Trace	0.1	0.2	0.2	0.1	Trace
Galactose	—	—	—	0.9	0.9	1.5	1.2	0.3	0.1
Arabinose	—	—	—	Trace	0.2	0.3	0.2	Trace	Trace
Oligosaccharides (including sucrose)	4.0	5.0	5.0	0.7	0.8	2.2	2.2	0.4	0.3
Fructans	6.4	5.3	4.8	0.1	0.1	0.1	0.1	0.1	0.1
Mannitol	—	—	—	3.6	3.7	1.9	1.1	4.3	4.1
Cellulose	27.5	26.5	26.0	28.6	28.8	28.6	29.5	30.1	30.2
Lignin	4.0	4.4	4.3	4.4	4.4	4.4	4.2	4.7	4.1
Formic acid	—	—	0.92	Nil	Nil	0.5	0.6	0.1	Trace
Acetic acid	—	—	—	2.4	3.0	0.8	0.9	3.2	3.6
Propionic acid	—	—	—	0.03	0.05	0.08	0.05	0.18	0.17
Butyric acid	—	—	—	0.06	0.12	0.06	0.10	0.17	0.14
Lactic acid	—	—	—	5.9	8.2	4.3	4.8	11.1	10.2
Succinic acid	—	—	—	Nil	Nil	Nil	Nil	Trace	Trace
Ethanol	—	—	—	0.64	0.61	0.61	0.68	1.2	1.2
pH after maceration	6.08	6.21	4.93	4.18	4.29	4.39	4.45	3.94	3.94
Buffering capacity (b.c.) mequiv./100 g of d.m.	35	32	35	89	98	54	62	137	112

[a] W=Wilted; F=formic acid-treated; U=unwilted.

TABLE 2. Microbiological assay

Sample	Bacterial count[a] (no. of organisms/g of fresh material) YEA	TA	MA	GM	LM	pH	% Ethanol in d.m.	% w.s.c. in d.m.
Grass								
Uncut grass, 8 June, 8 am	1.2×10^3	100	<10	10^3	<10	6.20	—	19.3
Forage harvested grass, 8 June, 8.05 am	2.2×10^6	366	30	10^3	<10	—	—	—
Wilted grass, 9 June, 9 am	2.9×10^6	1.7×10^4	30	10^3	<10	—	—	—
Wilted grass, 9 June 3 pm	4.5×10^6	1.1×10^5	<10	10^3	<10	—	—	—
Forage harvested wilted grass, 9 June, 4 pm	4.6×10^6	7.2×10^5	<10	10^3	<10	6.21	—	16.8
Silage[b]								
After 1 day, silo A	6.5×10^8	1.0×10^7	373	10^4	<10	5.91	—	17.9
After 1 day, silo C	5.3×10^5	1.0×10^7	<10	10^3	<10	5.42	—	20.9
After 2 days, silo A	2.8×10^8	1.3×10^7	26	10^4	<10	5.00	—	10.3
After 2 days, silo C	4.9×10^5	1.0×10^5	103	10^4	<10	5.11	—	20.0
After 3 days, silo A	1.7×10^9	7.3×10^6	46	10^5	<10	4.85	0.5	—
After 3 days, silo C	3.1×10^6	1.4×10^6	293	10^3	<10	5.44	0.6	—
After 4 days, silo A	5.2×10^8	8.5×10^8	4.5×10^3	10^3	<10	4.80	0.5	9.8
After 4 days, silo C	1.1×10^8	1.1×10^8	1.0×10^4	10^3	<10	5.24	0.3	22.2
After 8 days, silo A	3.2×10^8	3.9×10^8	4.6×10^4	10^2	<10	4.60	0.4	9.7
After 8 days, silo C	1.2×10^8	2.1×10^8	9.8×10^4	10^2	<10	4.91	0.6	15.7
After 23 days, silo A	2.0×10^8	0.4×10^8	1.4×10^5	<10	<10	4.34	1.4	5.8
After 23 days, silo C	4.6×10^8	4.8×10^8	2.8×10^6	<10	<10	4.61	1.5	7.5
After 34 days, silo A	7.6×10^6	4.8×10^7	1.9×10^5	<10	<10	4.28	1.2	4.9
After 34 days, silo C	1.3×10^8	6.4×10^7	0.9×10^5	<10	<10	4.36	0.8	11.4
After 71 days, silo A	9.0×10^7	8.0×10^7	10^2	<10	<10	4.18	1.0	3.8
After 71 days, silo C	1.4×10^9	1.7×10^9	10^3	<10	<10	4.38	0.8	4.9
After 149 days, silo A	2.6×10^6	1.2×10^6	<10	<10	<10	4.18	0.6	4.8
After 156 days, silo C	2.5×10^5	1.6×10^7	6.4×10^3	<10	<10	4.39	0.6	15.1

[a] YEA = Yeast extract agar (total count—all organisms)
MA = Malt agar (yeasts and fungi)
LM = Lactate medium (lactate fermenters).
TA = Tween agar (lactic acid bacteria)
GM = Gelatin medium (proteolytic Clostridia)

[b] Silage samples, except final ones, were taken from the side ports using a corer.

3.2. Composition

The composition of the grasses and silages is shown in Table 1. The d.m. content of the acid-treated wilted grass (36%) was slightly higher than that of the untreated material (32.3%). The main differences in the d.m. components are seen in the carbohydrate and organic acid fractions, the soluble carbohydrate percentages being higher and the acetic and lactic acid percentages lower in the formic acid-treated silages compared with the untreated materials. These differences in acid content are also reflected in the pH values of the silages. The unwilted silages are typical of such material, being of low pH (3.94), of relatively high lactic acid content (10.7%) and containing little residual soluble carbohydrate (1.2%).

3.3. Microbiological assay

Core samples were taken from side ports in silos A and C after 1, 2, 3, 4, 8, 23, 34 and 71 days and results of microbial counts for these together with counts on original grass samples and final silages are given, using five different media, in Table 2. Although total counts of micro-organisms were consistently lower in the formic acid-treated silages there was no evidence from the Tween acetate agar counts, with the possible exception of the sample obtained after 2 days, to indicate that the activities of lactic acid bacteria were lower in the formic acid-treated material than in the untreated herbage. Microbial proteolytic and lactate fermenting activities were negligible.

3.4. Losses

The losses of d.m. are shown in Table 3. The total d.m. loss is made up of two fractions—fermentation plus oxidation and waste material. Using the bag and marker technique referred to earlier it was possible to calculate the fermentation plus oxidation losses from the well-preserved edible material separately and these are also reported in Table 3.

TABLE 3. Losses during ensilage

	Silo			
	A (W)[a]	B (W)	C (WF)	D (WF)
Herbage d.m. ensiled (kg)	280.9	280.9	281.5	281.5
Edible silage d.m. removed (kg)	244.0	238.9	218.6	231.3
Waste d.m. removed (kg)	14.7	20.6	29.4	24.1
[b] Fermentation plus oxidation loss in edible silage (%)	3.0	3.0	7.9	7.5
[c] Waste loss (%)	10.4	12.4	15.6	11.1
Total loss (%)	13.4	15.4	23.5	18.6
Loss of total N (%)	+3.0	+1.1	3.6	4.2
Loss of w.s.c. (%)	72.5	73.6	25.2	22.6

[a] W=Wilted; F=formic acid-treated.

$$[b] = 100 - \frac{\text{kg d.m. removed as edible silage}}{\text{kg grass d.m. ensiled} - \text{kg grass d.m. to form waste}} \times 100.$$

$$[c] = \frac{\text{kg grass d.m. to form waste}}{\text{kg grass d.m. ensiled}} \times 100.$$

3.5. Laboratory silos

The analytical results of the laboratory silages are shown in graph form in Figure 2.

The pH values of the laboratory wilted silages followed a similar pattern to those found for the cored samples taken from the large silos. The pH values of the treated and untreated fresh silages in the final stages were similar.

The w.s.c. components were consistently higher in the formic acid-treated wilted silages than in the untreated material. In the unwilted silages, formic acid had only a slight effect in preserving w.s.c.

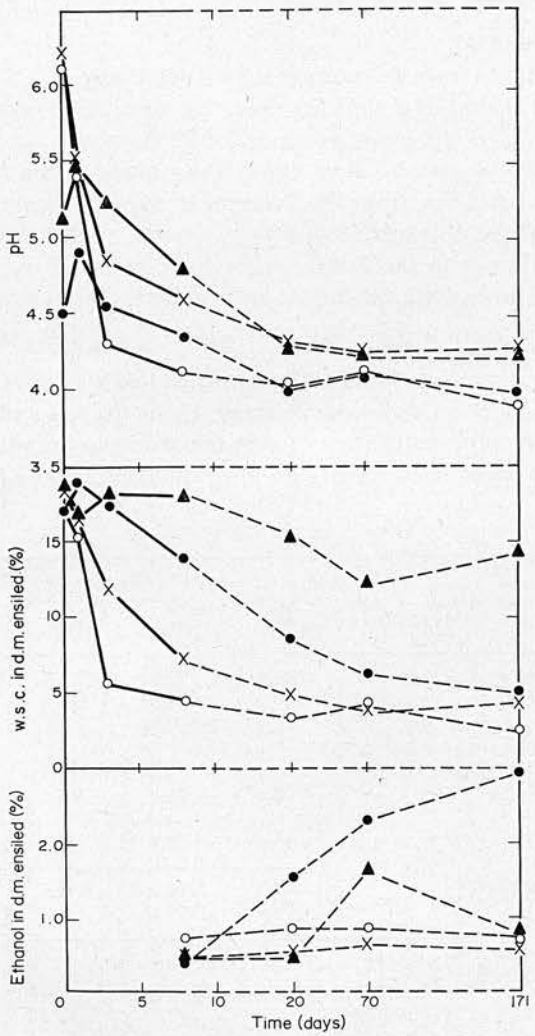

Figure 2. pH, water-soluble carbohydrates and ethanol values for laboratory tube silos. (○) Fresh; (●) fresh plus Add-F; (×) wilted; (▲) wilted plus Add-F.

The ethanol values for the acid-treated fresh herbage were about four times those obtained for the other silages in the final samples.

4. Discussion

In previous studies[8] using 14.5% d.m. fresh timothy/meadow fescue grass, Add-F applied at the commercially recommended level (0.27%) resulted in an immediate pH fall to 4.75. In the present experiment using 36% d.m. wilted ryegrass the application of 0.39% Add-F caused the pH to fall to 4.93. With the exception of d.m., w.s.c. and non-protein nitrogen (n.p.n.) the untreated and acid-treated wilted herbages were similar in composition to the fresh grass. The increase in n.p.n. during wilting confirms the results obtained in a previous experiment[3] that proteolytic enzymes become active immediately after harvesting.

Accepting that temperature rise within the silo is a direct measure of rate of respiration, formic acid apparently inhibited to some extent respiration in the early stages of ensiling; this finding agrees with the results of other workers.[12] The temperature peak in Figure 1 corresponds to the time of application of consolidation weights on day 3, confirming that adequate compression of the herbage mass in the silo is an effective method of controlling temperature in wilted material.

The silage analytical results (Table 1) indicate that formic acid effectively restricted fermentation in the wilted herbage. The lower lactate, acetate, buffering capacity (b.c.) values and higher w.s.c. components support this conclusion.

The carbohydrate components of the acid-treated silages are particularly interesting in that the residual w.s.c. were high—about 15%—and of these only 1.8% could be accounted for in terms of monomers derived from heteropolysaccharides. High residual w.s.c. values (>15%) have been reported in earlier studies[3] with heavily wilted Italian ryegrass of 47% d.m. Mannitol, a product of the action of heterofermentative lactic acid bacteria on fructose,[3] was present in all silages, although the concentration in the formic acid-treated silages was less than half that found in the non-treated wilted and fresh silages.

Formic acid had no obvious effect in preventing proteolysis during ensilage although it appeared to have a slight effect in inhibiting deamination. In previous studies using fresh grass,[8] formic acid had a beneficial effect in preventing amino-acid catabolism. The fermentation in all silages was clearly dominated by lactic acid bacteria, there being only small amounts of products derived from clostridial activity.

The microbiological data (Table 2) obtained from cored samples confirm that proteolytic clostridia and lactate fermenters were relatively inactive in the wilted silages, although surprisingly counts on the Tween acetate agar, a medium specific for lactic acid bacteria, were similar for both the untreated and acid-treated silages. In the later cored samples, taken after 8, 23, 34 and 71 days, there is some evidence from the w.s.c. values, that these were not truly representative of the silage mass and the bacteriological counts should be interpreted accordingly. In these later samples, silage had to be removed via ports which had been opened for previous sampling and although care was taken to avoid sampling from identical areas within the silo, some aerobiosis in the vicinity of the port may have affected the fermentation pattern.

The lactic acid bacteria increased on the grass during wilting and also immediately after forage harvesting. This last finding confirms the results of previous studies.[13] With the exception of the first day's samples and particularly in the final sample, yeast counts were higher in the formic acid-treated silages. These high counts can be explained by the findings of concomitant studies[14] showing that formic acid is less inhibitory to yeasts than to lactic acid bacteria. The possibility of yeasts accounting for losses in formic acid during ensilage by using it as a C-source is being investigated. The products of yeast fermentation are ethanol and carbon dioxide. There is however no apparent evidence from the ethanol contents of the silages of excessive yeast activity in the treated silages, the concentrations being similar (0.6 to 0.7%) in the four wilted silages. Ethanol, however, is also a by-product of the fermentation of glucose by heterofermentative lactic acid bacteria[3] and it is possible that the ethanol in the untreated silages was derived via this last pathway rather than from yeast fermentation. The mannitol values lend support to this hypothesis.

The losses data in Table 3 are more difficult to interpret. Assuming restricted bacterial activity in the formic acid-treated silages it would be reasonable to expect lower d.m. losses during ensilage of the treated herbage, but this did not occur. The higher waste losses obtained in the treated silages indicate that oxidation in the surface layers was greater than in the untreated silages. This finding confirms previous results that formic acid does not have any beneficial effect in preventing the formation of surface waste.[8,12] When d.m. losses in the edible material alone are considered, the values for the untreated silage (3%) are very low compared with the acid-treated material (8%). A more active yeast fermentation with correspondingly high gaseous (CO_2) loss may be a partial explanation for this finding.

The analytical results using laboratory tube silos are similar to those obtained using the large silos and confirm that formic acid preserves the w.s.c. in the wilted grass. The high ethanol values obtained for the acid-treated fresh grass silages indicate that a yeast-type fermentation occurred in this material.

The significance of these differences in composition between the silages and in particular the importance of high residual w.s.c. in the acid-treated wilted silage to the ruminant animal will be reported in a later paper.[15]

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